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Some Unsolved Problems Concerning the Pathogenesis of Human Deficiency Disease Syndromes

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IT IS a great pleasure for me to join in this Symposium honoring Dr. Sydenstricker. Until recently I thought that Dr. Sydenstricker and I had only one common interest: nutritional diseases. Last week I found another: we have each studied with the same eminent teacher, Dr. Edwards A. Park. When I told Dr. Park I was coming to this celebration he related a story of Dr. Sydenstricker's student days. It seems that Dr. Park had a group of three students, each of whom afterwards attained eminence in medicine: V. P. Sydenstricker, T. M. Rivers, and D. W. Atchley. One day he showed this group of students a Negro child with enlarged epitrochlear glands. They all thought it was a case of tuberculosis. Dr. Park maintained that it was syphilis. The next day Dr. Park had decided that the students were right. When he told them so they said that they too had changed their minds about the diagnosis and now thought the case was one of syphilis. What it really was we will never know!

Today a number of human deficiency disease syndromes are recognized to be caused by a lack of one or more indispensable nutrients, whether these be inorganic elements, amino acids, vitamins, etc. Such syndromes may be conveniently classified as in Table I. Most of them result from a lack of exogenous nutrients, i.e., those which must be furnished from outside sources. Here, the deficiency may result from an inadequate intake or because certain conditioning factors may interfere with the ingestion, absorption, storage, assimilation and excretion, or increased needs may be demonstrated. In addition to these exogenous deficiency states, attention must always be given to those which may result from lack of endogenous nutrients. These include any or all of the essential metabolites manufactured by the organism from the indispensable nutrients and would therefore include a long list of protein, carbohydrate, or lipid materials. A familiar example of one such nutrient is, of course, glucose, a deficiency of which leads to the hypoglycemic syndrome.

The classification of the diseases shown in Table I is based on etiology; hence, one might assume that specific therapy is available for each. In the main this is true. Yet another

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TABLE I

Deficiency Disease Syndromes in the Human

1. The inorganic elements
   - (A) The low-sodium syndrome
   - (B) The hypokalemic syndrome
   - (C) Tetany
   - (D) Iron-deficiency anemia
   - (E) Endemic goiter
2. Amino acids and protein
   - (A) Hunger edema
   - (B) Pellagra
   - (C) Kwashiorkor
   - (D) Nutritional liver disease
3. Carbohydrate
   - (A) The hypoglycemic syndrome
4. Vitamins
   - (A) Xerophthalmia (hypovitaminosis A)
   - (B) Rickets and osteomalacia
   - (C) Tocopherol deficiency
   - (D) Scurvy
   - (E) The beriberi syndrome
   - (F) The Wernicke syndrome
   - (G) Pernicious anemia
   - (H) Non-Addisonian megaloblastic anemias
5. Other
   - (A) The malabsorption syndrome
   - (B) Dental caries
   - (C) Nutritional melalgia
   - (D) Blood coagulation defects

aspect is equally clear; in most cases we do not understand the pathogenesis of the disease state which is being treated. Each of the syndromes listed in Table I has certain clinical and pathologic criteria which characterize it more or less specifically. However, when one attempts to explain in a coherent fashion the deranged mechanisms responsible for such abnormal clinical and pathologic findings, such fundamental information is meager indeed.

If one examines the history of nutrition during the past half century or more, he can trace the development of this state of affairs; for the accumulation of our knowledge of human deficiency diseases has in general followed a fairly definite pattern.[1] First, the clinical and pathologic manifestations of the disease were delineated. Sometimes, for instance in the case of scurvy, this was followed by the recognition of some material or groups of substances which appeared to exert a beneficial effect. The great advance, however, came when the syndrome, or something akin to it, could be reproduced in the experimental animal. When this had been accomplished, information concerning some material which might have a protective effect was not long in forthcoming so that an active substance could be isolated, crystallized, identified, and finally synthesized. Ultimately, its biochemical role might be elucidated.

Unfortunately, in this story of progress many things remained to be studied concerning the original syndrome which had been responsible for all the subsequent investigations. One has only to consult the current index volumes of, let us say, *Nutrition Abstracts and Reviews*, to be aware of the paucity of articles on the pathogenesis of pellagra, beriberi, goiter, etc, during recent years. Hence, there are many unsolved problems concerning the nature of the syndromes listed in Table I which still require study; we need a reorientation of emphasis from the laboratory to study human disease in its natural state. This leads us to another fact which stands out when one examines the history of nutrition during the past four or five decades. Nutritionists have been concerned with single, i.e., pure, deficiency states. This is a logical outcome of the studies of foodstuffs such as rice, corn, or wheat by McCollum and others during the early part of this century which led to the isolation and designation of a host of essential nutrients and naturally focused attention on investigations based on diets adequate in all respects save for a single substance. Now with an adequate baseline of information dealing with the biochemical and anatomic effects of deficiencies of single nutrients we are in a position to unravel some of the complexities of multiple deficiency states, which is the way nutritional disease occurs naturally.

In our laboratory, we have recently begun a program which actually takes up where McCollum and others left off around 1915. It is our purpose to reproduce and study in experimental animals certain human disease syndromes which have occurred in the past and continue to be observed today in various parts of the world. We have believed that our understanding of these syndromes in man is inadequate; consequently, if these could be reproduced in experimental animals, we would be in a position to understand their pathogenesis better.

We have chosen corn and polished rice as the two basic foodstuffs with which to produce nutritional disease. The consumption of corn, of course, has been intimately concerned with the prevalence of pellagra for two centuries. More recently in certain areas of the world this cereal grain has come to be associated with the syndrome of kwashiorkor. Corn is deficient in protein, particularly with respect to quality since its content of tryptophan and lysine is low. It is low in calcium, magnesium, sodium, and iodine. Certain vitamins are deficient, particularly ascorbic acid and niacin; a portion of the latter which is present is unavailable. Rice, too, contains an insufficient amount of quality protein, is lacking certain minerals and in particular is deficient in thiamine and certain other vitamins of the B-group as well as ascorbic acid. From data which have already been reported in several species by others it was expected that nutritional disease would appear when corn and rice were fed in unsupplemented form to experimental animals, though we were unprepared to predict precisely what would happen.

At this time, we should like to present some of our preliminary findings since they illustrate certain problems which are pertinent to those encountered in studies of human nutritional disease.

KWASHIORKOR

When young growing monkeys are fed whole ground unenriched corn supplemented with ascorbic acid, a definite syndrome results.[2] In brief, this consists of failure to grow and slowly progressive weight loss. When liver tissue is studied by biopsy very early, that is, after about a month, fatty infiltration of the periportal portions of the lobules is seen. The fat content of the liver then increases so that by the time the animal dies the total lipid concentra-

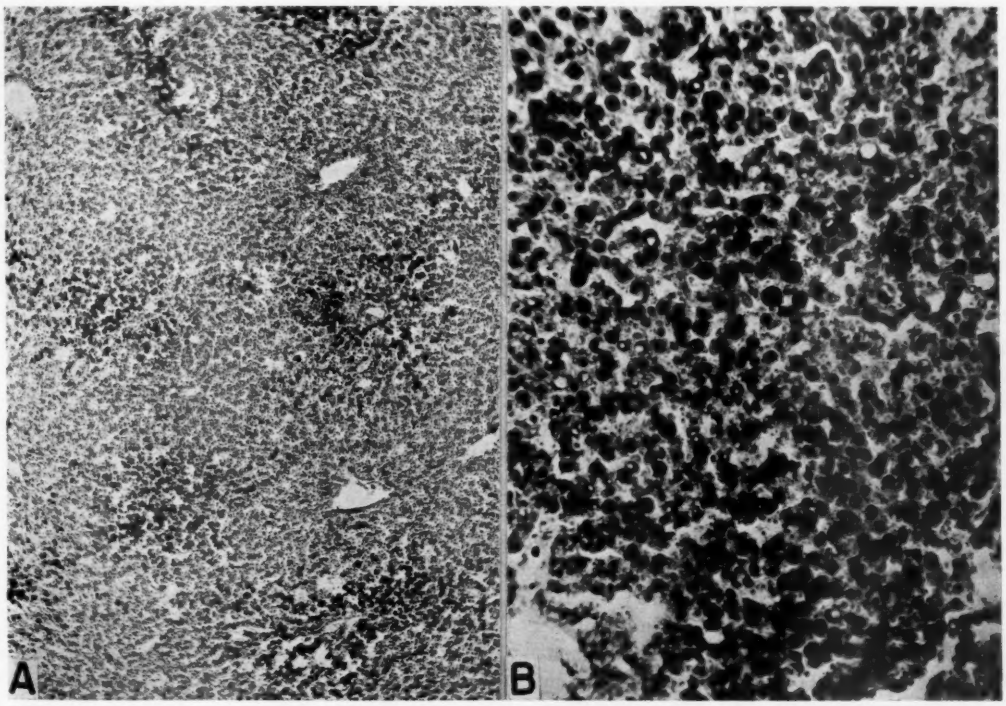

Fig. 1. Liver, monkey. (A) (x35). From monkey on corn diet for 43 weeks. This animal had lost 38 per cent of its initial weight. Note periportal accumulation of fat. (B) (x100). Liver from monkey coming to autopsy after 41 weeks on the diet, having lost 25 per cent of his initial body weight. Fat is excessive and accounts for 68 per cent of the dry weight of the liver.

tion may be more than 20 per cent of the total wet weight. No changes in the color of the hair have been observed although in several animals alopecia was prominent. As time goes on the animals become less active and sit huddled in their cages. The plasma protein concentrations begin to decrease slowly. The reduction is due to a diminution in the albumin fraction. Clinical edema, as evidenced by swelling of the periorbital tissue and genitalia has been observed.

At autopsy, depending on the duration of the deficient state, one finds fatty infiltration of the liver of varying degrees. This is corroborated by the lipid content as determined by alcohol-ether extraction. Sections of the skin reveal that the collagen fibers are separated from one another; this confirms the edema noted clinically. No specific alterations have been noted in the pancreas. The other tissues show varying degrees of atrophy which coincide with the severity of weight loss which was observed clinically. When the skeletal tissues are studied, a definite decrease in maturation is found; that is, the osteones are smaller and fewer in number than they should be. No evidence of excessive osteoid is found.

This, in brief, represents a syndrome which has been observed in varying degrees in animals which have come to autopsy. Others are still being studied. It is of interest that the clinical and pathologic picture has many similarities to kwashiorkor as it is seen throughout the world today.[3] Most workers who have observed kwashiorkor in children would doubtless agree that it can be characterized as follows: failure of growth, psychic changes, alterations in the hair, dermatitis, enlargement of the liver due to fatty infiltration, edema, hypoproteinemia as a result of hypoalbuminemia and diarrhea. A number of these characteristics have been observed in the monkeys which have just been described. The outstanding discrepancy is the lack of involvement of the skin and hair in the experimental animal. It would appear, therefore, that means are at hand whereby a syndrome resembling kwashiorkor can be reproduced in the laboratory, which may enable us to gain more information concerning the naturally occurring disease.

It was most interesting to have a syndrome such as this develop on a corn diet, for we had fully expected something more reminiscent of pellagra to occur. Corn has, of course, been associated with pellagra for many years. Hence, one might have expected that these monkeys fed maize might have exhibited skin lesions, more prominent gastrointestinal disturbances and, perhaps, even neurologic involvement. The unpredictable outcome of the effects of the corn diet only serves to emphasize our complete ignorance concerning the pathogenesis of pellagra. To a deficiency of what essential or essentials may the dermatitis be ascribed? How do the lesions of the esophagus, colon, and vagina come about? What is the pathogenesis of the neurologic changes? What is the basis for the fatty infiltration, usually periportal, of the liver? These and other questions have never been answered and furnish some of the many unsolved problems in nutritional disease.

The complexity of the problem can best be exemplified by listing certain characteristically clinical manifestations of pellagra, together with certain nutrients which have been shown to produce these alterations under experimental circumstances in man.

(a) *Erythematous or scaling dermatitis:* tryptophan-niacin;[4] pyridoxine;[5] biotin.[6]

(b) *Seborrhea:* riboflavin;[7] tryptophan-niacin;[4] pyridoxine.[5]

(c) *Scrotal dermatitis:* riboflavin.[7]

(d) *Vaginitis:* tryptophan-niacin.[4]

(e) *Cheilosis:* riboflavin;[7] tryptophan-niacin;[4] pyridoxine.[5]

(f) *Glossitis:* iron;[8] tryptophan-niacin;[4] pyridoxine;[5] folacin;[9] vitamin B_{12};[9] biotin.[6]

GOITER

Another important human syndrome, which has widespread prevalence is endemic goiter. It is estimated that throughout the world today there are over 30 million cases of goiter. Few areas are spared and in many regions the incidence may be extremely high, even among young children. Our own attention has recently been directed to goiter because of enlargement of the thyroid glands of rats which had been subsisting on corn diets for prolonged

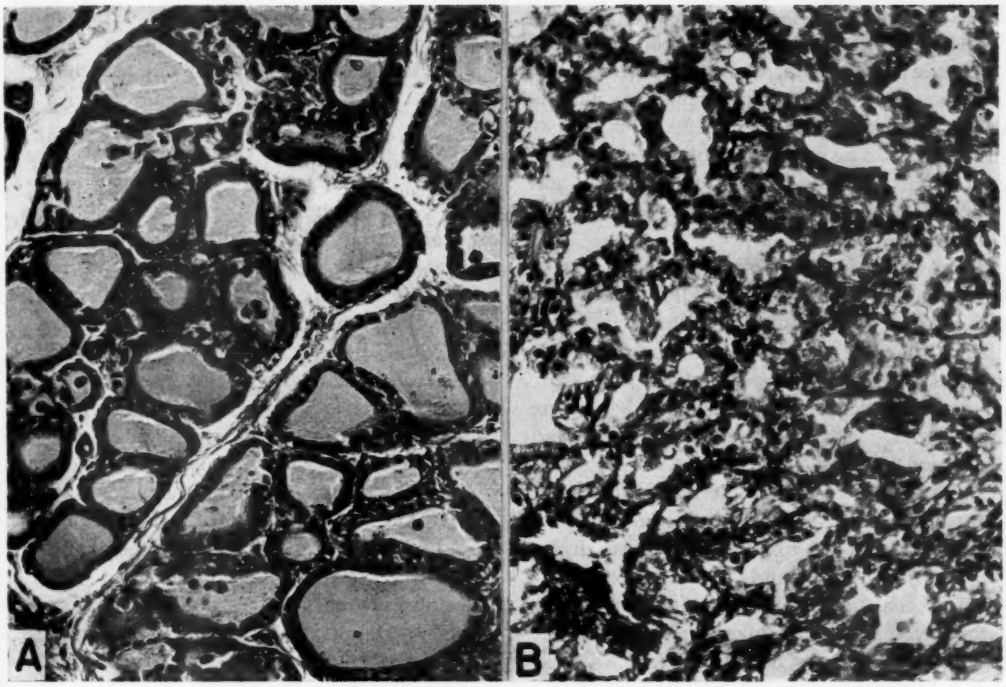

Fig. 2. Thyroid, rat. (A) (x200). Normal control. Note size of follicles which are well filled with colloid. (B (x200). Animal on diet of whole corn. Note small follicles with large cells and absence of colloid.

periods.[10] Similar increase in size of thyroids of monkeys fed corn has not been observed. In retrospect the appearance of goiter in our rats is not too unexpected, since one of the main constituents of the goiter-producing diet which was employed by Remington[11] in early studies of goiter was corn.

The point we wish to make here is that endemic goiter represents another one of the unsolved problems of human nutrition. Pathologically, the endemic goiter consists of enlarged thyroid follicles distended with colloid. Everyone is familiar with the hypothesis proposed by Marine,[12] which states that iodine deficiency leads to hyperplasia of thyroid epithelium. With the reintroduction of iodine into the deficient organism, the nearest state to normal which the thyroid can attain is a structure composed of follicles lined by flattened epithelium and filled with large amounts of colloid. In other words, the initial stage is hyperplastic goiter, while colloid goiter represents an attempt to attain normalcy. Marine based this hypothesis on studies correlating the iodine content of the gland with its histologic appearance. Observations on the geographic distribution of iodine with respect to goiter incidence, as well as prophylactic tests which were carried out in Akron, Ohio, from 1916 to 1920[13] seemed to settle the pathogenesis of endemic goiter. One would naturally have expected that the entire cycle of normal to hyperplastic goiter to colloid goiter could have been worked out easily in suitable experimental animals in the laboratory. Such, however, has not yet been attained. To be sure, the development of thyroid hyperplasia as a result of diets of low-iodine content has been described by several investigators. But production of typical colloid goiter has not been realized in the laboratory. As a result of the great impetus to studies of the thyroid gland, which followed the introduction of antithyroid compounds 15 years ago and the current availability of radioactive iodine, more light has been thrown on the pathogenesis of goiter. The studies of

Stanbury[14] and others have contributed much, yet the problem of tracing the biochemical and anatomic development of colloid goiter in the experimental animal still remains. Factors other than iodine deficiency may, of course, play a role. The possible effects of goiterogens, of minerals, such as calcium and fluoride, and of still undiscovered factors remain to be fully elucidated.

From our own studies of thyroid enlargement in the rat on corn diets it would appear that this goiter is due to iodine deficiency, since supplementing the grain with potassium iodide prevents its development. The histologic change in the thyroid gland is one of extreme hyperplasia. No vestige of excessive colloid deposition is seen. That an interference with the elaboration of thyroid active principle has occurred is brought out by enlargement of the hypophysis and the presence of typical thyrotropic cells in this gland. Why colloid goiter has not been successfully produced in the laboratory remains an open question. Perhaps the proper species has not been utilized yet. Would it not be advisable to study the problem in animals such as dogs and swine, in which goiter has been reported to occur naturally?

BERIBERI

The third human syndrome which poses many problems as to its pathogenesis is beriberi, a disease characterized to varying degrees by heart failure, polyneuritis and edema. This malady has been recognized for centuries in those areas where the main dietary staple is rice. Since, even today, half of the peoples of the world live on rice, the problem of clinical or subclinical beriberi continues to be a very real one. It is not necessary to review the history of beriberi and the isolation of thiamine, the genesis of which goes back to Eijkman's description of "eine beriberi-ahnliche Krankheit der Hühner."[15] It is sufficient to indicate that thiamine deficiency in many mammalian species has led to lesions in the heart and central nervous system. No alterations have been found in the peripheral nerves except in birds as originally described by Eijkman. The

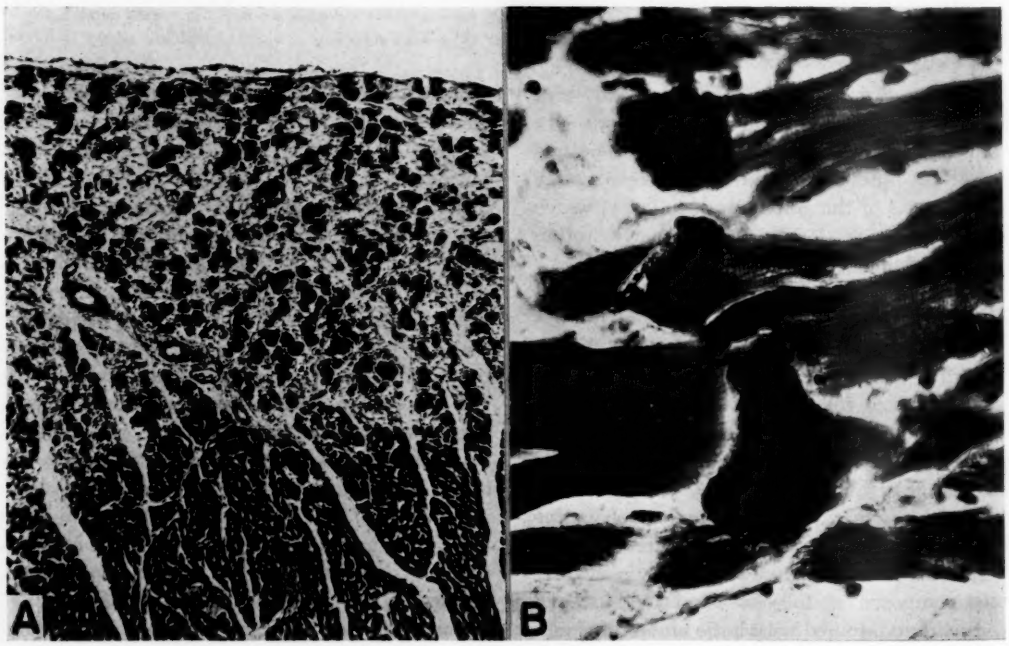

Fig. 3. Heart, monkey. (A) (x70). Area of necrosis beneath epicardium from animal on rice diet for seven weeks. Note absence of inflammatory cells. (B) (x500). Note normal striated myocardial fibers which end abruptly in foci of necrosis. Portions of the fibers have completely disappeared.

pathogenesis of the cardiac manifestations of beriberi is doubtless related to thiamine deficiency since cardiac lesions have been produced in various species on thiamine-deficient diets.[9] On the other hand, both clinical trials in man and studies in experimental animals have not clarified the pathogenesis of the neurologic changes save those which appear to be associated with the Wernicke syndrome.[9]

To gain more information, if possible, on the pathogenesis of beriberi, monkeys have been maintained for various periods of time on a diet of polished, unenriched rice which is supplemented only with ascorbic acid. No evidence of neurologic impairment, either central or peripheral, has been recognized. On the other hand, manifestations of cardiac damage have been dramatic and severe. Unlike the lesions in the heart which we and others have seen in various species on thiamine-deficient diets, the changes in our rice-fed monkeys have been characterized by widespread fresh necroses, so recent that virtually no cellular infiltration yet has appeared. The fulminating course of this experimental disease is reminiscent of the rapid progression of the disease in infancy, though, of course, sudden death in adults has long been well-recognized.

Here again we are faced with a question: what is the basis for the neurologic manifestations of beriberi which classically have been described as a "polyneuritis"? What obviously is needed is intensive study at autopsy of humans dying of the disease where it is still endemic. Virtually no such studies have been reported since the early observations which were based on cases coming to autopsy in Japan, China, and the Dutch East Indies. Almost 50 years have elapsed since then. Modern methods of study by competent neuropathologists would seem eminently worthwhile today.

These then are a few of the unsolved problems in human deficiency disease. Today the nutrients essential for man appear to have been delineated. Yet how they interact and how their reduction or lack precisely affect the organism so as to give rise to specific clinical and pathologic changes which comprise human deficiency disease states are questions which have yet to be solved. There is still much to be done!

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On the Role of Antibiotics in Nutrition and Metabolism

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THE role of antibiotics in nutrition is centered —if not exclusively, then certainly with obvious preference—around the intestinal flora and specifically around the interaction between antibiotics, intestinal flora, and the host organism.

Man and animals live in symbiosis with microbes; in particular, the intestinal flora may act as a modifying environmental factor, and as such may influence growth, development, and the metabolic processes of the host organism. It was Escherich[1,2] and his followers[3] who first established the fact that the composition of the intestinal flora is primarily influenced by the food ingested and in the final analysis by the milieu in the intestinal lumen, acting as culture medium, with selective capacity for bacterial inhabitants of the intestinal tract.

On the nutrition and metabolism of the host organism the intestinal flora may exert its effect in different directions: (a) Beneficially such as through synthesis of essential nutrients (mainly of micronutrients) or through transformation of undigestible or poorly digestible food constituents, for example, cellulose and other polysaccharides, especially in ruminants; (b) in a more harmful direction, as by utilizing nutrients and thus withholding them from the host organism, or by the formation of toxic metabolites which adversely effect the host organism.

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INTESTINAL FLORA AS SOURCE OF VITAMINS

Intestinal flora as a possible source of vitamins, synthesized by bacteria, was first discussed by Cooper as early as in 1914.[4] The first most convincing proof for the synthesizing ability of the intestinal flora for a vitamin in man was furnished by the study of prothrombin levels as an index of vitamin K activity in the newborn infant.[5] Prothrombin levels in the serum, after an initial drop following delivery, show a sudden increase, after the first three to five days of life apparently synchronized with the establishment of a functioning bacterial flora in the intestine.

There exists a wide literature on the synthesis of vitamins by the intestinal flora in experimental animals. It is outside the scope of this presentation to review the extensive experimental material in detail. It should suffice to call attention to a few basic observations gained by experiments on animals. Fridericia[6] described spontaneous recovery in rats kept on a diet deficient in the vitamin B complex without the addition of vitamin-containing supplements. The exact nature of this phenomenon, called refection, is not yet determined. It is still an open question whether it is due to intestinal absorption of vitamins synthesized by the bacteria or to coprophagy. The best and unequivocal evidence, not only for the role of intestinal bacterial synthesis of vitamins, but also for their nutritional efficacy, was first furnished by experiments on rats and later on other animal species fed purified diets supplemented with various sulfonamides.[7–11] In addition to vitamin K, biotin, folic acid, and to some extent perhaps nicotinic acid, may be furnished by intestinal bacteria and their intestinal syn-

thesis be suppressed by sulfonamides. Experiments with sulfonamides on human volunteers[12–15] seemed to indicate that such synthesis of various vitamins or vitamin-like compounds may occur also in man through the metabolic activity of the intestinal flora. However, these observations were largely inconclusive with regard to the availability of these bacterial products for the host organism.

The first observation on the beneficial effect of antibacterial substances for the nutritional state of animals was first reported by Moore and co-workers in 1946. They found that succinylsulfathiazole and streptomycin increased the growth of chicks on a purified diet.

ANTIBIOTICS

Growth Promoting Effect

Greater and general interest in antibiotics as useful dietary supplements in practical animal husbandry was stimulated and spread widely only later, since 1950, through the studies of Jukes and his associates on the growth-promoting effect of antibiotics in animals.[16] At present antibiotics, such as penicillin, the tetracyclin group, and others, are widely used as supplements in commercial animal feeds. A very extensive literature on antibiotics as growth stimulants and dietary adjuvants has accumulated during the last eight or nine years.[16,17] Nevertheless, the mechanism by which the antibiotics produce their effects on nutrition is still an open problem. It is generally related to the action of antibiotics on the intestinal bacteria. "When antibiotics are administered, the total numbers of bacteria in samples of the intestinal contents characteristically decrease for a few days and then increase above the original levels. Perhaps antibiotics suppress certain bacterial forms which are inhibitory not only to the growth of the host, but also to the growth of some of the other intestinal bacteria."[16]

Seen from this angle it is not surprising that observations on the nutritional effect of antibiotics vary widely not only in different laboratories, but often in the same laboratory at various times. It has been claimed that the intestinal flora may show equal variations. Growth promotion by antibiotics would then occur only when bacteria are present in the gut which in themselves retard growth of the host and may be eliminated under the influence of antibiotics.[16,17] It was in accord with this assumption that the growth-promoting effect of antibiotics was found to disappear or be diminished when chicks were raised in clean quarters.[18] In addition, the promotion of growth in animals under the influence of particular antibiotics may be related to the relief of subclinical or clinically mild intestinal infections. Clostridium has been widely named[17] as the bacterial species around which the growth-promoting effect may be centered. A particular strain of Clostridium has recently been isolated in the National Institute for Research in Dairying in Reading (England) which was found to be present in chicks which responded with enhanced growth to the administration of antibiotics and was found to be absent in refractory animals.[19]

As expected, germ-free animals have shown no positive response to antibiotics. The absence of any growth-promotion in germ-free turkey poults, compared with the conventional controls fed the identical ration, was especially conclusively demonstrated by Forbes, Supplee and Combs in yet unpublished experiments carried out in the Germ-Free Research Laboratory of the Walter Reed Army Institute of Research and the University of Maryland.

Difficulties of proper controls have made studies on the growth-promoting effect of antibiotics in infants and children even less easy to assess than those carried out on animals. The most impressive positive results were reported by Scrimshaw and his associates[20,21] on Mayan children 7 to 12 years of age living in the Guatemalan highlands and subsisting on diets low in animal protein. Chlortetracycline exerted a pronounced effect on the increases in height of the children. This effect was observed in the spring months and was related to the suppression of seasonal infections in the children. The response to penicillin was variable and not as consistent as with tetracycline.

Use in Elimination of Toxic Factors

Antibiotics may exert their effect on the intestinal flora either through elimination of

toxic factors or by sparing beneficial nutrients. Suppression of infection is considered in this context as tantamount to the elimination of toxic factors. More conclusive evidence for the harmful role of toxic metabolites produced by intestinal bacteria was furnished by clinical observations on patients with severe liver disease, near decompensation. In such patients Sherlock and her associates found methionine given in very high doses orally (8–20 g/day) to be distinctly toxic. Chlortetracycline given together with methionine to the same patients prevented the development of toxic manifestations.[22] The development of coma in patients with hepatic decompensation following a high-protein diet has been generally related to the absorption of nitrogenous toxic metabolic products of bacterial action.[23] Hence, the recommendation of antibiotics in the treatment of hepatic coma.[24]

Sparing Effects

The sparing effect by antibiotics concerning a beneficial nutrient is well demonstrated in animal experiments in which antibiotics, such as chlortetracycline and penicillin were used to delay the development of experimental dietary necrosis of the liver.[25]

In one experiment (Table I) 40 rats received first only the basal experimental diet. A second group of 20 rats received chlortetracycline (5 mg daily) and a third group of 20 rats penicillin, in form of a basic, poorly soluble compound (Bicillin 5 mg daily) as supplements to the basal experimental diet. At the end of 26 experimental days ten rats in the control group died from acute massive necrosis. Of the remaining 30 rats in this group, 11 animals received Bicillin® (5 mg daily) from the 26th experimental day on. Eleven animals starting on the same day received chlortetracycline (5 mg daily). The remaining eight rats in this group were left on the unsupplemented basal diet.

Rats receiving supplements of chlortetracycline or Bicillin from the start were subdivided into two groups each. One subgroup originally receiving chlortetracycline was shifted after 44 days to Bicillin, the other subgroup was kept on chlortetracycline. The same scheme was applied to the group receiving Bicillin, with the difference that the change took place on the 42nd experimental day.

The results obtained seem to indicate (Table I) that the effect of antibiotics is not simply antibacterial, and further, that the development of bacterial resistance may not play, at least in this particular case, an important role if any in determining the duration of the prevention of massive hepatic necrosis.

These conclusions are best supported by the beneficial results of Bicillin and chlortetracycline which are significantly more impressive when administered during the whole duration of the experiment. This is in contrast with the group of animals (C, D, Table I) in which the supplemented diet was begun after the rats were kept for about four weeks on the unsupplemented basal experimental diet. These observations are in closest accord with the hy-

TABLE I

The Effect of Continuous, Delayed, or Alternating Administration of Antibiotics on the Development of Massive Dietary Hepatic Necrosis

Group	Supplement	Number of rats	Died with hepatic necrosis	Average survival time *days*	Survived 150 days without hepatic necrosis
A	None	10	10	32±0, 8	—
B	None	8	8	34±1, 3	—
C	Nothing for 26 days, then Bicillin	11	10	44±7, 6	1
D	Nothing for 26 days, then chlortetracycline	11	9	50±9, 0	2
E	Chlortetracycline	10	4	54±8, 5	6
F	Chlortetracycline for 44 days, then Bicillin	10	4	63±5, 0	6
G	Bicillin	10	2	42±0, 0	8
H	Bicillin for 42 days, then chlortetracycline	10	2	112±2, 0	8

Average starting weight of animals in all groups was 49 grams.

pothesis that necrosis develops after the experimental animals have become depleted of protective food constituents. Antibiotics appear to prolong this period of depletion. In groups C and D (Table I) the depletion probably has already progressed too far; consecutive administration of antibiotics may not have much effect in staving off the early development of massive hepatic necrosis. The experiments with alternating administration of chlortetracycline and Bicillin furnished results no better than those with continuous use of one given antibiotic (group E and F or G and H, respectively). These results[25] not only do not favor the toxic etiology as the only cause of experimental hepatic necrosis, but at the same time leave the mode of action of the antibiotics, and in particular their role in delaying the state of "depletion," unexplained.

An impressive illustration for the sparing of a nutrient through the action of antibiotics in man is furnished by the effect of antibiotics on the metabolism of choline. When a relatively large amount of choline is ingested by normal persons about 60 per cent appears in the urine as total trimethylamine, mostly within 24 hours.[26] Oral administration of chlortetracycline, oxytetracycline and penicillin or sulfaphthalidine, but not intravenous chlortetracycline, causes a considerable reduction in the urinary excretion of trimethylamine after simultaneous ingestion of a test dose of choline, as the result of a diminished intestinal degradation of choline to trimethylamine by intestinal bacteria. With continuous administration of antibiotics, such as penicillin, the antimicrobial effect disappears within one or two weeks indicating refractiveness of the bacteria.[27] Thus, in this particular case, antibiotics, may help temporarily at least to increase the available amount of ingested vitamin by protecting it from bacterial degradation in the intestine. Such sparing of a vitamin could easily be mistaken for its intestinal synthesis under the influence of the antimicrobial agent in question.

Sparing or intestinal synthesis of vitamins must be the explanation for the beneficial effect of some antibiotics in rats fed rations deficient in B vitamins, such as pyridoxine or riboflavin or pantothenic acid. This positive effect of antibiotics observed by Daft and his associates[28,29] is not seen in all the animals treated, but on the other hand, if present, it may last for many months.

Metabolic Effects

Antibiotics may also produce secondary metabolic effects, which are not of strictly nutritional nature. In our laboratory it has been found that rats fed a diet producing necrosis of the liver, or the same ration supplemented with cystine or vitamin E excreted large amounts of ether-soluble acids, especially methylmalonic acid in their urine. Rats on the same diet supplemented with chlortetracycline and penicillin excreted only small amounts of these acids. This effect persisted as long as the antibiotics were given and is the first long-term persistent *in vivo* effect noted of chlortetracycline and penicillin.[30] The urinary excretion of methylmalonic acid was increased in rats fed the necrogenic basal ration after supplementation with valine. This increase after administration of valine was not observed in rats which were kept on the necrogenic diet and received supplements of chlortetracycline. In contrast, in liver perfusion experiments the production of methylmalonic acid from valine (or from propionate) took place regardless whether chlortetracycline was added to the perfusion mixture or not. These experiments seem to indicate that chlortetracycline acts through the bacterial flora of the intestine, and not primarily through the metabolism of the liver.[31]

Use in Posthemorrhagic Shock

Recently it has been claimed, especially by Fine and his group,[32,33] that irreversible posthemorrhagic shock, as produced experimentally on animals, may be beneficially influenced by preventive medication with antibiotics. The further assumption has been made that antibiotics may eliminate intestinal bacteria which produce endotoxins of primary etiologic importance in the chain of events leading to irreversible shock. If this were the case, animals raised under germ-free conditions should be resistant to the same posthemorrhagic insult, which in conventional animals end in fatal shock. In unpublished experiments, carried

out independently in two laboratories, at Notre Dame University and at the Walter Reed Army Institute of Research, no distinct difference was observed in the behavior of conventional and germ-free rats when they were exposed to the conditions of irreversible posthemorrhagic shock. Germ-free rats have developed shock of apparently the same intensity and in about the same time, as the conventional controls. Whether the unavoidable possible admixture of traces of endotoxin and bacterial bodies in the sterilized semisynthetic ration fed to both groups may play a role in the production of shock under germ-free conditions, only further special studies will be able to decide. In this connection it would be important to know whether the beneficial effect of some antibiotics on the prevention of irreversible posthemorrhagic shock may be duplicated in germfree animals. If this were the case, a direct metabolic effect of the antibiotics in question would have to be postulated.

It appears to be obvious that antibiotics may interfere in both a beneficial or harmful manner with the nutritional state and with several metabolic reactions of the host organism. The observations illustrate clearly the interdependence of nutrition, metabolism, and infection.

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Recent Advances in Intravenous Fat Alimentation

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THE INCREASING advances in medical and surgical technics available today make the search for intravenous fluids capable of providing complete parenteral alimentation to the patient over a given period of time, ever critical. Those solutions presently available to the clinician for intravenous feeding fall short of the ideal goal. By complete alimentation is meant a regimen which provides adequate calories, carbohydrate, protein, fat, minerals, and vitamins. At the present time, caloric needs afforded best by fat, are most difficult to furnish. Numerous studies have shown quite well that amino acids, for example, will not appreciably contribute to protein synthesis unless adequate calories are given. Apparently, the protein precursors will be used for energy purposes in the absence of adequate calories. Thus intravenous solutions supplying calorigenically rich fat would be ideal for such purposes. These realizations have been the basis for numerous investigations over the years into the development of a utilizable intravenous fat emulsion.

The contributions to the development of such a preparation are multitudinous and cannot be adequately acknowledged in this report. Freeman has detailed much of the early developments in his excellent monograph on the subject.[1] The problems of composition, stability, animal studies, etc., are monuments to the diligence and ingeniousness of many experimentors. Literally hundreds of oils and emulsifiers have been mixed in varying combinations searching for a safe and practical product. Certainly Stare and his co-workers at the Harvard School of Public Health deserve credit for their exhaustive efforts in this regard. Finally cottonseed oil emulsified with soybean phosphatide in a high pressure homogenizer, stabilized with a synthetic polymer, pluronic F68, and rendered isotonic with 4 per cent dextrose was found most satisfactory. Then followed literally thousands of clinical trials in human beings. It soon became obvious that this preparation, although superior to previously tested emulsions, was still accompanied by an incidence of adverse side reactions too great to allow for general practical usefulness. Thus in our series of 229 infusions in 110 patients there was an over-all reaction rate of approximately 50 per cent per patient.[2] The reactions consisted of severe back pain, fever, chills, dyspnea, cyanosis, and rarely, acute shock. Fever alone occurred in approximately 30 per cent of the subjects.

Several observations suggested that the soybean phosphatide which was used might be responsible for the high reaction rate. This fraction has since been purified and in January 1956 the presently available emulsion was ready for clinical testing. Our original studies with the improved emulsion were performed on 129 patients receiving 298 infusions and have been recently extended by us as well as many others. These observations have shown that with the prominent exception of

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fever, all other adverse reactions have been virtually eliminated from the single infusion.[2,3] Fever, albeit mild, still appears in approximately 30 per cent of patients receiving intravenous fat emulsions.

The persistence of fever as an untoward reaction, even though all other side effects had been eliminated with the purification of the phosphatide, led us into a study of the nature of the delayed, usually mild, and clinically unimpressive febrile reaction. Early studies by our group had failed to incriminate pyrogens, particle size or *in vivo* hemolysis as a cause of the fever.[4] During the course of observations on the manner in which individuals cleared the infused fat emulsion from their blood, it became apparent that a highly significant correlation existed between the rate of clearance and the febrile reaction.[5] Those patients in whom the infused fat was removed from the blood very slowly did not develop fever, whereas in those subjects in whom fever did occur the fat disappeared rapidly. It was an interesting clinical observation that the "sicker" the patient, the more likely it was that fever would occur and that rapid clearance would be found. Not all patients who were "fast clearers" necessarily developed fever, however. These data were interpreted as suggesting that fever results in susceptible persons from endogenous heat produced by rapidly cleared and metabolized fat. The alternate hypothesis that there is a "pyrogenic substance" associated with the fat which is more rapidly released during the process of fast clearing cannot be denied. Further efforts at "cleaning up" the oil may finally resolve this problem.

In an effort to establish the assumption that rapid clearing is evidence of rapid oxidation, blood ketone levels were measured in a group of hospitalized subjects and correlated with clearing rates.[6] Much to our surprise, only minor rises occurred in blood ketones following the infusion of 600 ml of a 15 per cent cottonseed oil emulsion. These rises were so small that no significant correlation between them and clearing could be made. Since other evidence indicates that the fat is metabolized, the two-carbon fragment released by oxidation of the fatty acid chain presumably moves immediately into the citric acid cycle and the opportunity for condensation into acetoacetate is not afforded.

The variation in the manner in which the fat is removed from the blood, noted between individuals, as well as within the same individual under varying conditions, is an interesting phenomenon. The physiologic mechanism for clearing is poorly understood at the moment. Studies from our laboratory have shown that clearing-factor enzyme or lipoprotein lipase does not play a major role intravascularly.[7] There is reason to believe that a major portion of the infused fat actually leaves the blood stream as particulate fat—the chylomicron. Its ultimate fate thereafter remains to be elucidated, but it is probable that it is handled in much the same manner as is ingested fat.

It would seem, therefore, that there is available a fat emulsion readily acceptable to the majority of patients who need it, provided it is administered for a short period of time. However, the question of its safety and utility after many repeated infusions remained. Several cases[8,9] have been reported of patients receiving multiple fat emulsions in whom a rather characteristic moderately severe, clinical syndrome appeared. These unfortunate subjects developed a febrile illness associated with hepatosplenomegaly, anemia, jaundice, abdominal discomfort and bleeding tendencies. The nature or cause of this illness has not been adequately explained.

We have had the opportunity of observing two such long-term reactions to intravenous fat emulsion therapy. One such patient was a 44-year-old white woman admitted to the Psychosomatic Ward of the Cincinnati General Hospital because of a long history of functional gastrointestinal symptoms, severe psychoneurosis, hysterical syncope, weight loss, and diarrhea. After an intensive diagnostic workup failed to reveal any significant organic disease, she was given 600 ml of Lipomul®* intravenously each day for 24 days. After 22

* Lipomul, Upjohn, is a 15 per cent cottonseed oil emulsion, containing 1.2 g per cent soybean phosphatide, 3 per cent Pluronic F68 and 4 per cent dextrose and was kindly furnished by Dr. E. A. Hawk, The Upjohn Company, Kalamazoo, Michigan.

uneventful infusions the patient began to complain of vague abdominal pain anorexia. She developed low grade afternoon fever. By the 24th infusion vomiting began which was rapidly followed by progressive fever, hepatomegaly, and lethargy. The intravenous fat therapy was discontinued. The fever progressively rose until it reached 105° F (Fig. 1). At this point intravenous hydrocortisone was instituted which was followed by a lysis of the fever and a rapid restoration toward normal in all clinical manifestations. The various clinical and laboratory data are shown in Figures 1 to 4. These, along with observations from other patients receiving multiple fat infusions can be summarized as follows:

Hemogram (Fig. 2): Mild, progressive anemia occurred. There was an associated reticulocytosis; however, studies of fecal urobilinogen and red cell survival with Cr^{51} failed to demonstrate a hemolytic process. Bone marrow studies before and after the fat were essentially normal as was red cell uptake of Fe^{59}. Blood loss except from venipuncture could not be established. There was no change in the leukocytes nor was there a significant drop in the circulating thrombocytes.

Liver Function (Fig. 3): No change occurred in any liver function test except for the BSP retention which progressively increased. This has been noted frequently by others but is always reversible with discontinuation of the fat. This may represent "clogging" of the reticulo-endothelial cells by the particulate fat, a well-recognized occurrence following fat emulsions. Biopsy of the liver in one patient with this syndrome failed to demonstrate excessive fat deposition in the liver parenchyma but did show small nonspecific microgranulomas unassociated with fatty material or pigment.

Lipid Studies (Fig. 4): There is no evidence that persistent hyperlipemia occurred during the daily administration of the lipomul. Although a very slight increase in total fatty acids was noted, there was no change in the fasting optical density of the serum. There was some decrease in fat tolerance as measured by the clearing test, but this did not progress after the first week. The rather marked rise in esterified cholesterol has not been a routine finding in other patients, and may well be related to the low pre-infusion cholesterol values in this patient. Our experience with multiple infusions to date shows that the cholesterol changes may be quite variable.

Coagulation Studies: As will be noted from Table I there are two important changes noticeable. One is the hypercoagulability which accompanies each individual infusion. The other is the hypocoagulability noted at the time of the acute febrile illness. Although this patient

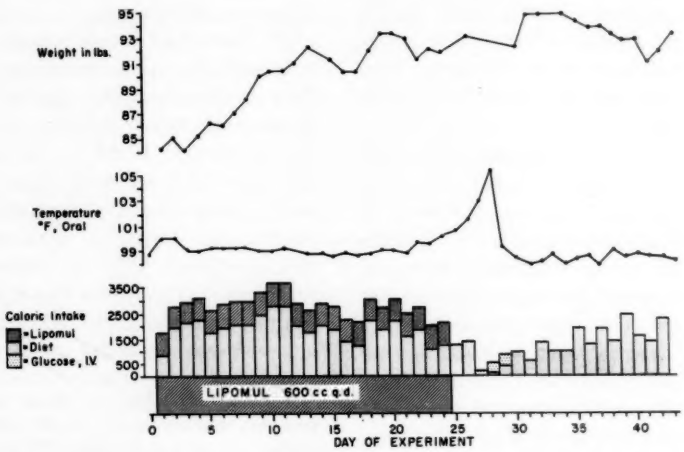

Fig. 1. Effects of daily administration of fat emulsion on weight and dietary intake.

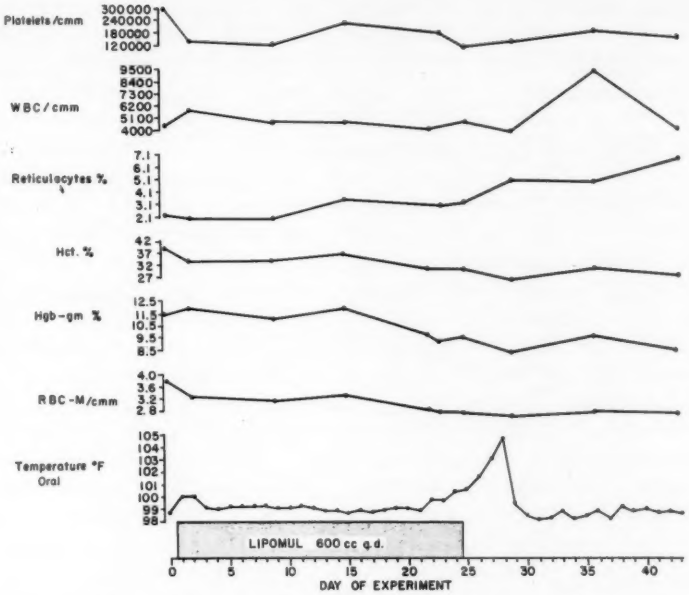

Fig. 2. Effects of daily administration of fat emulsion on the blood count.

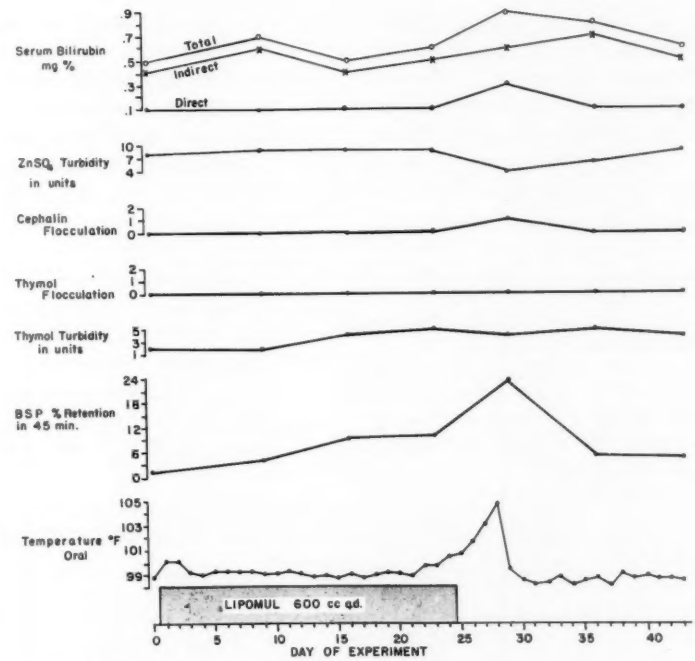

Fig. 3. Effects of daily administration of fat emulsion on liver function tests.

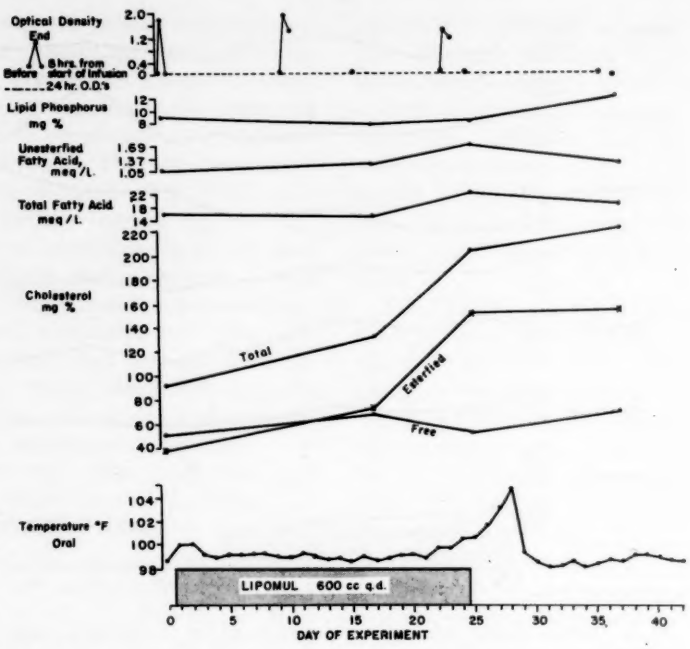

Fig. 4. Effects of daily administration of fat emulsion on serum lipids.

did not bleed, it was the clinical impression from observations of needle punctures that a definite bleeding tendency existed. This was borne out by the prolonged clotting time and markedly depressed prothrombin as measured by the TAMe assay.[10] This combination of hypercoagulability associated with individual infusions and a gradually decreasing concentration of prothrombin after repeated infusions has been a constant finding in our patients. Further work is being pursued along these lines in collaboration with Dr. Helen Glueck, but at the present the hypothesis is offered that the soybean phosphatide in the emulsion acts as

TABLE I

EFFECTS OF INTRAVENOUS FAT ON COAGULATION MECHANISMS

Date	7-31-57	8-6-57		8-12-57		8-20-57		8-23-57		9-3-57	9-6-57	9-17-57
		Before	After	Before	After	Before	After	Before	After			
Siliconized Lee White Clotting Time												
1st Tube	4 min.	14 min.	6min.	9 min.	——	——	6min.	9 min.	6 min.	22 min.	8 min.	6 min.
2nd Tube	——	19 min.	8min.	14 min.	——	——	12min.	14min.	——	41 min.	12 min.	12 min.
3rd Tube	26min.	25min.	——	20min.	——	——	12min.	18min.	——	60min.	16min.	17min.
Plasma Prothrombin Time												
Control	11.4 sec.	——	13 sec.	13.0sec.	——	——	13.0sec.	12.7sec.	——	11.6sec.	11.1sec.	10.5sec.
Patient	11.9 sec.	——	11.5sec.	11.5sec.	——	——	12.5sec.	11.2sec.	12.6sec.	16.0sec.	13.7sec.	11.2 sec.
	(95%		(100%	(100%			(100%	(100%	(100%	(57%	(70%	(97%
Serum Prothrombin Time												
28° Serum												
20 min. inc.	11.3 sec.	——	<60sec.	8.8sec.	——	——	8.5sec.	8.7sec.	10.9sec.	10.5sec.	10.2sec.	9.5sec.
40 min. inc.	132 sec.	——	<60sec.	9.9 sec.	——	——	9.5sec.	11.0sec.	10.9sec.	11.9sec.	12.1 sec.	9.8sec.
60 min. inc.	60.2sec.	——	<60sec.	22.4sec.	——	——	17sec.	13.6sec.	48.8sec.	11.6sec.	13.4sec.	15.2 sec.
Plasma TAMe Assays	35u/ml	——	38 u/ml	32 u/ml	——	——	28u/ml	32 u/ml	39 u/ml	10 u/ml	28 u/ml	49 u/ml
Serum TAMe Assays												
20 min.	27 u/ml	——	1 u/ml	20 u/ml	——	——	5.0 u/ml	34 u/ml	7 u/ml	4 u/ml	0	36 u/ml
40 min.	12 u/ml	——	1 u/ml	11 u/ml	——	——	5.0 u/ml	5 u/ml	7 u/ml	0	0	13 u/ml
60 min.	8 u/ml	——	2 u/ml	6 u/ml	——	——	8.2 u/ml	6 u/ml	6 u/ml	0	0	7 u/ml
Factor V	normal		normal	normal			normal	normal	normal	slight fall	normal	normal
Factor VII	normal		normal	normal			normal	normal	normal	normal	normal	normal

a thromboplastic material which promotes the increased coagulability. Repeated episodes of the hypercoagulability state gradually deplete the body of prothrombin which cannot be adequately replenished. Whether or not this theory will prove to be true, only future work will disclose. It would seem, however, that the effect of repeated infusions of fat on the coagulation processes is a fertile area for further research.

As yet there is no adequate explanation of the severe clinical syndrome precipitated by repeated administration of intravenous fat emulsions. There is not enough experience as yet to predict how many patients or which ones will develop the reaction, nor how many infusions would be required to produce it. It should be mentioned that in most instances in which the syndrome has developed, a large caloric intake has been provided the patients. The latter have been, for the most part, experimental or volunteer subjects who were not particularly ill. This has led to the hypothesis that the syndrome may represent an "overloading phenomenon" with fat. Our studies of the serum failed to demonstrate hyperlipemia nor did the liver biopsy reveal excessive lipid in the liver, thus there is no proof of this attractive hypothesis. The obvious clinical similarity between this reaction and idiopathic hyperlipemia gave impetus to the above suggestion.

The prompt response to hydrocortisone in the patient herein described suggests that hypersensitivity should be an etiologic consideration. At this moment there is nothing to prove or disprove such a possibility. Our findings related to the clotting abnormalities certainly do not explain the entire picture, but may give some insight into one of the more important clinical manifestations. Further experience will be necessary before more definitive statements can be made and steps taken to prevent this important reaction to repeated infusions of fat emulsions.

SUMMARY

It would appear that the presently available fat emulsion is a safe and practical product for short-term administration. However, if it is to be used for repeated and long-term therapy, the possibility of the development of the described febrile syndrome should be appreciated. As yet no test is known which will alert the clinician to its imminence. The advisability of intermittent rather than persistent therapy is suggested.

There are many problems yet to be met in the general field. The present phosphatide-stabilized emulsion is unstable to electrolytes or amino acids so that a "complete" emulsion is not feasible. Non-phosphatide emulsions prepared with synthetic emulsifiers are stable to these supplements but as yet are attended with high reaction rates in humans.[11] In addition, lipomul is "broken" by freezing so that emulsions resistant to environmental extremes would be desirable. Dehydrated preparations have been studied and their development will be watched with interest.

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Niacin-Tryptophan Relationships in Man and Niacin Requirement

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PARTICIPATION in this Symposium honoring Dr. Virgil Sydenstricker is a real privilege and pleasure. It seems particularly suitable to discuss certain aspects of niacin metabolism as Dr. Sydenstricker and his associates have made many contributions to this field.

Knowledge of niacin-tryptophan relationships in man will be reviewed briefly in historical perspective before discussing current research. It is of considerable interest that astute clinical observations of 200 years ago have been validated and explained by recent scientific experiments.

In the first descriptions of pellagra in the eighteenth century, both Casal and Frapoli[1] noted the association of the disease with a poor diet in which corn (maize) was one of the principal foods. Casal[2] even described the good effects of a "milk diet." In the early nineteenth century, Fanzaga and Marzari[1] suggested that pellagra was due to protein inanition and that maize was deleterious because of its low nitrogen content. Roussel,[2] in his treatise on pellagra in 1845, emphasized the importance of diet in therapy. His rules "of the alimentary regimen of the pellagrous" included exclusion of maize of poor quality and bringing into the diet "a progressively increasing proportion of animal substances beginning with the milk diet."

Early in the present century Funk[3] suggested that pellagra was due to vitamin deficiency. Another etiologic concept at this time, held particularly by observers of pellagra in Egypt, was that this disease was related to lack of an essential amino acid, probably tryptophan.[3] In the course of Goldberger's classic investigations of the etiology of pellagra, he showed that the disease could be prevented by increasing the intake of fresh animal and leguminous protein foods.[4] Subsequently, he induced pellagra in 6 of 11 subjects who subsisted for seven months on a diet similar to that consumed by persons with endemic pellagra.[5] In continued search for the pellagra preventive factor, Goldberger[6] conducted experiments which linked tryptophan to the pellagra problem. One of the corn proteins, zein, had been known since the early 1900's to be of poor biologic value, being low in lysine and tryptophan. These amino acids were administered to pellagrous subjects and improvement followed tryptophan administration. At this time, Goldberger suggested that pellagra might be due to amino acid deficiency. However, further research with food sources of the pellagra-preventive factor made vitamin deficiency seem a more likely possibility and the idea of a relationship to protein received less attention.

Concurrent with the studies of Goldberger, Voegtlin[7] demonstrated that a proper diet containing milk, eggs and meat was an essential aspect of the treatment of pellagra. He assumed that there might be a deficiency of certain vitamins and a deficiency of certain amino acids in the pellagragenic diet.

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Subsequent to these studies, pellagra was treated with a good diet, yeast and, when it became available, with liver extract. Then, in 1937, Elvehjem and his associates[8] demonstrated that niacin and its amide cured "black tongue" in dogs, a condition previously shown to be the canine analogue of pellagra. Following this discovery, niacin was found to be effective in the treatment of pellagra by a number of investigators.[9] In 1938, Schmidt and Sydenstricker[10] attempted to prevent relapse of chronic pellagra with nicotinic acid, 100 mg being given twice weekly. This quantity proved insufficient and the importance of diet and other accessory factors in the prevention of the pellagra syndrome was emphasized.

Although niacin had been demonstrated to be the pellagra-preventive vitamin, certain aspects of the occurrence of pellagra remained unexplained. Diets in some areas of the world in which pellagra was rare were found to contain less niacin than did corn diets in areas in which pellagra was common. Furthermore, certain foods known to be effective in the prevention of pellagra, such as milk, were found to be low in niacin.

TRYPTOPHAN

In 1945, Krehl and associates[11] observed growth retardation in rats when corn was included in large amounts in a low protein ration. This retardation was corrected by the administration of niacin or of the amino acid, tryptophan. It had been shown previously that the rat could synthesize niacin and that the synthesis appeared to be related to the protein level of the diet. In 1946, Rosen and associates[12] reported that the administration of tryptophan to rats was followed by a marked increase in the urinary excretion of niacin metabolites. This was confirmed by Singal, Briggs, Sydenstricker and Littlejohn[13] who found that after tryptophan was given, there was an increased excretion of nicotinic acid, a methylated derivative of nicotinic acid and an unidentified substance which was converted to nicotinic acid by acid but not by alkaline hydrolysis. They suggested that this unidentified substance might be quinolinic acid. In 1947, Sarett and Goldsmith[14] and Perlzweig and associates[15] showed that administration of tryptophan to human subjects led to an increased excretion of N^1-methylnicotinamide (N^1-Me) in the urine. These experiments in rats and in man indicated that tryptophan might be an important precursor of niacin.

Since this time, tryptophan has been shown to be effective in preventing and curing nicotinic acid deficiency in many animal species.[16] Pellagra was found to respond satisfactorily to treatment with large doses of tryptophan by Sarett and Goldsmith[17] and by Vilter, Bean and their associates.[18] Administration of tryptophan to human subjects is followed by an increase in the excretion of N^1-Me, the pyridone of N^1-Me and quinolinic acid.[14–17, 19]

It was thought by some that tryptophan might exert its effect through the intestinal flora. However, further investigation showed that injection of tryptophan caused an immediate increase in the urinary output of niacin derivatives in the rat[20] and in man.[21] Formation of niacin from tryptophan was found to take place in the absence of the entire intestinal tract[22]; it also occurred in liver slices.[23]

TRYPTOPHAN CONVERSION

The mechanism of conversion of tryptophan to niacin was elucidated to a large extent by studies in mutant fungi.[24] Intermediates in this conversion in neurospora were found to be kynurenine, 3-hydroxykynurenine and 3-hydroxyanthranilic acid. Henderson[25] found that administration of tryptophan or 3-hydroxyanthranilic acid to rats led to an increase in quinolinic acid excretion. He hypothesized that this substance was an intermediate in the conversion of tryptophan to niacin. However, the activity of quinolinic acid on a molar basis was found to be much lower than that of niacin or 3-hydroxyanthranilic acid. Accordingly, Krehl, Bonner and Yanofsky[26] suggested that quinolinic acid might be an alternate end product (Fig. 1). In human subjects, oral administration of quinolinic acid led to an increased excretion

of niacin metabolites but to a less extent than might have been anticipated had it been in the main pathway of conversion of tryptophan to niacin.[19]

Heidelberger and associates[27] proved conclusively by isotope experiments that tryptophan was converted to niacin in the rat. They showed that carbon atom 3 in the indole ring of tryptophan became the carboxyl carbon atom of nicotinic acid. Henderson and Hankes[28] studied the metabolic fate of tryptophan labeled with C^{14} in the benzine ring or in the α carbon atom in adult rats. With ring-labeled tryptophan, the distribution of C^{14} in the quinolinic acid excreted in the urine was in good agreement with theoretical values for conversion through the sequence: kynurenine, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid. Urinary N^1-Me had a specific activity which suggested a 5-fold dilution with body pools of tryptophan, pyridine nucleotides and other precursors.

Another feature of the conversion of tryptophan to niacin is of interest. Pyridoxal phosphate functions as a coenzyme with kynureninase in the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid.[29] In vitamin B_6 deficiency, kynurenine is diverted from this pathway to the formation of xanthurenic acid. Snyderman and associates[30] reported impairment in the conversion of tryptophan to niacin compounds in experimentally induced vitamin B_6 deficiency in infants.

Studies of the extent of conversion of tryptophan to niacin in man have been dependent on measurement of urinary excretion of niacin derivatives after tryptophan administration. The principal niacin metabolites in human urine are N^1-methylnicotinamide (N^1-Me)[31] and N-methyl-2-pyridone-5-carboxamide (pyridone).[32] Administration of tryptophan leads to an increase in excretion of N^1-Me[14–17] and pyridone[33] that is roughly proportionate to the size of the dose.[16, 34] There is also an increase in quinolinic acid excretion.[17, 19] Price and associates[35] reported that the chief urinary metabolites of tryptophan in man are kynurenine, kynurenic acid and o-amino hippuric acid. Xanthurenic acid, acetylkynurenine and anthranilic acid glucuronide were relatively minor metabolites. Pyridone was excreted to the extent of 0.8 per cent of a single oral dose of 2 g of L-tryptophan.

Experiments were conducted in our laboratory[36] in 14 adult subjects on controlled diets, either high or low in niacin and tryptophan, and supplemented at intervals with 2 to 6 g of DL-tryptophan for periods of 10 days or more (1 subject received 1 g of L-tryptophan). The following percentages of administered tryptophan were excreted as metabolites: 0.4 per cent as N^1-Me, 1.5 per cent as pyridone and 0.4 per cent as quinolinic acid. In these studies, it was assumed that D-tryptophan was not converted to niacin compounds. Considerable evidence supports this assumption[17, 34] but it has not been proved.[37] The excretion of niacin metabolites after administration of 10 to 30 mg of niacinamide was also measured in these subjects. From these data, the per cent conversion of tryptophan to niacin on a molar basis was estimated. Conversion averaged 3.3 per cent with a range of 1.9 to 5.0 per cent. The amount of dietary tryptophan which appeared to be equivalent to 1 mg of niacin averaged 55.8 mg with a range of 33.7 to 86.3 mg. Horwitt and associates[38] estimated the quantity of tryptophan converted to niacin in several groups of subjects maintained for months on diets low in niacin and tryptophan. One group received a supplement of 100 mg L-tryptophan, another a supplement of 10 mg of niacin and two groups were unsupplemented. Calculation of the amount of tryptophan equivalent to 1 mg of niacin from urinary excretion of N^1-Me, for two weeks of these regimens, gave figures of 52 and 65 mg. During the study, one group of subjects received a supplement of 3 g of lactalbumin which furnished about 200 mg of tryptophan. Findings in this group indicated that approximately 60 mg of the tryptophan provided in lactalbumin was equivalent to 1 mg of niacin. The findings of Goldsmith and Horwitt in subjects receiving diets which varied greatly in tryptophan and niacin content agree remarkably well. Accordingly, it seems justifiable for practical purposes to assume a factor of

60 to 1 for estimation of the efficacy of dietary tryptophan as a niacin precursor.

HUMAN REQUIREMENTS OF TRYPTOPHAN

Prior to the discovery that tryptophan was converted to niacin compounds, it had not been possible to determine the human requirement of this vitamin. Dann[39] estimated from a review of evidence obtained in dietary surveys that the minimum requirement of niacin for a 70 kg man was probably less than 10 mg. Frazier and Friedemann[2] calculated the protein and niacin content of pellagra-producing and pellagra-preventive diets and concluded that with a marginal diet containing corn products, the minimal niacin intake which would prevent pellagra approximated 7.5 mg daily. On a diet without corn, or one containing corn which was made adequate in some respects by the addition of large quantities of milk or milk products, the minimum need appeared to be about 4 mg daily.

After it was found that tryptophan was a precursor of niacin in man, experiments were initiated in our laboratory to determine niacin requirement and to elucidate the role of corn diets in the production of pellagra. Estimation of minimum tryptophan requirement by Rose[40] made possible formulation of diets containing little tryptophan in excess of that needed for maintenance of nitrogen balance. Diets were devised which were low in niacin and tryptophan and contained either corn or wheat as the principal cereal. A total of 19 long-term studies have been conducted in 15 adult human subjects, twelve females and three males.[33b,41] Niacin deficiency was induced in 15 of these experiments, the first signs appearing after one to several months. In 10 subjects deficiency was severe, in five it was mild. Practically all of the clinical features of pellagra were noted during the course of these studies although some subjects showed few or minimal lesions. Although the diets were supplemented with B vitamins other than niacin, cheilosis, angular fissures and nasolabial seborrhea were observed occasionally and healed after niacin administration. Twelve of the subjects who developed deficiency received diets in which corn was the principal cereal, three, diets in which wheat was substituted for corn. Deficiency tended to develop more rapidly and to be more severe with the corn than with the wheat diets; deficiency was less readily induced in males than in females.

The diets which resulted in niacin deficiency furnished 3.4 to 5.4 mg of niacin and 151 to 207 mg of tryptophan daily (Table I). Assuming that 60 mg of tryptophan is equivalent to 1 mg of niacin, the total niacin furnished by these pellagra-producing diets, or the "niacin equivalent" of the diets, ranged from 5.9 to 8.8 mg. The "niacin equivalent" of the diets in four subjects who failed to develop deficiency ranged from 7.4 to 10.6 mg.

Horwitt and associates[38] also conducted studies designed to determine human niacin requirement. The diets used by this group provided 5.2 to 7.0 mg of niacin and 238 to 318 mg of tryptophan. The diets contained no corn although 6 g of zein was included. Fifteen subjects received these diets for from 38 to 87 weeks without developing any clinical evidence of pellagra. Calculation of the total niacin furnished by these diets, including that potentially formed from tryptophan, gives a range of 9.2 to 12.3 mg (Table I).

From these data the minimal niacin required appeared to be in the neighborhood of 9 mg daily. However, calculation of the "niacin equivalent" of the diets used by Goldberger in the production of pellagra gives a total of 12.2 mg, if the tryptophan content of the diet is estimated as 330 mg.[38] Goldberger's diet furnished about 3,000 cal, while diets used by Horwitt supplied 2,070 to 2,760 cal and those of Goldsmith, 1,325 to 2,150 cal. These findings suggest that niacin requirement is related to caloric intake. Horwitt[38] concluded from an analysis of available data that the minimal amount of niacin which would prevent pellagra, including that formed from tryptophan and assuming a conversion figure of 60 to 1, was 4.4 mg/1,000 cal except with diets which supplied less than 2,000 cal in which instance 8.8 mg was required (Table I).

Goldsmith and associates[41] noted a relationship between niacin requirement and body size (Table I). Of twelve subjects who

TABLE I

Niacin and Tryptophan Intake in Relation to Experimental Production of Niacin Deficiency

Investigator	Number subjects	Niacin deficiency	Niacin in diet *mg*	Tryptophan in diet *mg*	Niacin equivalent* of diet *mg*	NE/* 1,000 cal *mg*	NE*/kg of body weight *mg*
Goldsmith	10	Severe	4.1–5.4	151–197	6.6–8.6	3.7–4.9†	0.11–0.18‡
Goldsmith	5	Mild	3.4–5.4	152–207	5.9–8.8	4.3–4.9†	0.12–0.20
Goldsmith	4	None	4.2–5.4	193–264	7.4–10.6	4.0–5.4	0.13–0.19§
Horwitt	15	None	5.2–7.0	238–318	9.2–12.3	4.4	0.15–0.21
Goldberger	11	Present in 6	6.7	330 (estimated)	12.2	4.1	0.14–0.22

* Niacin equivalent = dietary niacin plus 1/60 of dietary tryptophan.
† Subjects with ratios of 4.4 or above received less than 2,000 cal(1,600 to 1,900).
‡ Only one subject received more than 0.14 mg/kg.
§ Only one subject received less than 0.16 mg/kg.

received less than 0.15 mg "niacin equivalent" per kg of body weight, nine developed severe and two mild niacin deficiency, while one showed no abnormal findings. Of seven subjects who received 0.15 mg to 0.20 mg "niacin equivalent" per kilogram, three showed no evidence of deficiency, three mild deficiency and only one severe deficiency. Horwitt's fifteen subjects received 0.15 to 0.21 mg niacin equivalent per kilogram of body weight per day and none became deficient.

In other studies of Goldsmith and associates,[41a] subjects were given corn diets which supplied 200 mg of tryptophan and supplements of niacinamide were added in increasing or decreasing amounts. A significant change in the percentage of dietary niacin excreted in the urine as metabolites was observed when the niacin content of the diet approached 8 to 10 mg daily, suggesting that this level of niacin intake might be considered adequate. If it is assumed again that 60 mg of tryptophan will supply 1 mg of niacin, the "niacin equivalent" of these diets was about 11 to 13 mg.

Only one study of niacin requirement has been conducted in infants and none in children. Holt[42] maintained infants on a synthetic diet free of niacin and found that requirements were met, as judged by excretion of N^1-Me, when 15 per cent of the calories was supplied by casein. If the casein content of the diet was reduced to 10 per cent of the calories, excretion of N^1-Me decreased to a minimal value. The "niacin equivalent" of the 15 per cent casein diet, calculated as described above, would be 6 mg, that of the low-casein diet 4 mg.

"Niacin equivalents" have been calculated for some of the diets used by investigators in inducing pellagra prior to implication of niacin or tryptophan in the disease and also for diets used in testing the pellagra-preventive value of foods (Table II).[2,9] Values for

TABLE II

Niacin Equivalents of Pellagra-Producing and Pellagra-Preventive Diets

Pellagra-producing diets	Niacin equivalents
Unsupplemented	8.9–14 mg
Supplemented—preventive	10.4–22 mg
Supplemented—semipreventive	6.6–12 mg
Supplemented—not preventive	6.8–11 mg

"niacin equivalents" of the pellagra-producing diets of Goldberger, Ruffin and Smith, Spies, and Sydenstricker ranged from 8.9 to 14 mg daily. Such figures are presumably too high as no allowance was made for loss of nutrients in food preparation. Furthermore, available data on the tryptophan content of many foods are meager and average values are often based on widely varying analytic figures.[43] It is of interest to note that most investigators observed recovery of some subjects on these pellagra-producing diets.[2,9] Calculations of

the "niacin equivalent" of Goldberger's diets which were supplemented with certain foods and found to be pellagra-preventive[2] give values of 10.4 to 22 mg. Diets with supplements which were found to be semi-preventive furnished about 6.6 to 12 mg "niacin equivalent." Diets with supplementary foods which failed to prevent pellagra furnished 6.8 to 11 mg "niacin equivalent." Dietary data in a study of niacin requirement by Briggs, Singal and Sydenstricker[44] were also used for calculation of "niacin equivalent." Two subjects with signs of mild niacin deficiency were observed for 9 and 42 weeks without progression of lesions. These diets furnished about 9.3 and 8.0 mg of "niacin equivalent," respectively. In view of the inaccuracy of dietary calculations, there is remarkable agreement of these data with recent findings of Goldsmith and Horwitt.

OTHER FACTORS

From these studies of niacin-tryptophan relationships and niacin requirement, it is apparent that the pellagragenic effect of corn diets may be explained in large part by low niacin and tryptophan content. Other factors may play a minor role, however. In 1951, Kodicek[45] reported the presence of "bound" niacin in corn which was unavailable to the rat unless the corn had been subjected to alkaline hydrolysis. Laguna and Carpenter[46] noted that the growth depression in rats induced by high corn diets could be diminished by treatment of corn with lime. It has been suggested that release of "bound" niacin from corn by lime treatment, prior to incorporation in the diet, may explain the relatively low incidence of pellagra in areas such as Central America in which corn is prepared in this manner. In the only clinical study in which this hypothesis has been tested, experimental pellagra was produced as readily with lime-treated as with untreated corn.[41b] In this study, corn products furnished only 15 to 20 per cent of the diet whereas in Central America, corn products supply about 80 per cent of the caloric intake. Lime treatment might have an influence in the latter situation but this has not been investigated. Other explanations for the low incidence of pellagra in areas where corn is treated with lime should be considered, particularly the niacin and tryptophan furnished by the remainder of the diet. A recent study in our laboratory[34] indicates that coffee contributes a significant amount of niacin to the diet, yet ingestion of this beverage has never been considered in evaluating dietary niacin intake. A cup of coffee may furnish 1 to 3 mg of niacin depending on darkness of roast, amount of coffee used and method of preparation.

Several other possibilities merit consideration in discussing the pellagragenic effect of corn. This cereal may contain some toxic or inhibitory factor, as noted by Wooley[47] in experiments in mice. Available data suggest that if this factor has a role in human pellagra, it must be a minor one. Amino acid imbalance has been shown to influence niacin-tryptophan metabolism in animal experiments.[48] Such imbalance in corn diets has not been excluded as a factor in the genesis of pellagra in man.

SUMMARY

Pellagra has been known to be associated with diets high in corn and low in animal protein since it was first described two centuries ago. Recent research had elucidated this relationship, clarified the etiology of pellagra and made possible the estimation of human niacin requirement. Niacin was shown to be the pellagra-preventive vitamin in 1937. Some years later, the amino acid, tryptophan, was found to be a precursor of niacin in many animal species and in man. Administration of tryptophan is followed by an increase in urinary excretion of niacin metabolites. Tryptophan, when given in large doses, is effective in the treatment of pellagra. The pathway of conversion of tryptophan to niacin was elucidated by studies in neurospora; intermediate substances were found to be kynurenine, 3-hydroxykynurenine and 3-hydroxyanthranilic acid. Proof of a similar pathway of conversion in mammals was demonstrated by experiments with C^{14} labeled tryptophan.

Studies in normal adults have shown that an average of 3.3 per cent of administered

tryptophan is converted to niacin compounds. On a molar basis, approximately 60 mg of dietary tryptophan appears to be equivalent to 1 mg of niacin.

The minimum human requirement for niacin is about 9 to 12 mg daily in adults, including that formed from tryptophan. Requirement appears to be related to body size and to caloric intake. The close association of pellagra with diets high in corn can be explained in large part by the low niacin and tryptophan content of this cereal. Other factors may have a role in the pellagragenic effect of corn but appear to be of minor importance.

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The Significance of Amino Acid Imbalance in Nutrition

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AMINO ACID *imbalance* is the term commonly used to designate a relative deficiency of an essential amino acid resulting from an excess of one or more amino acids in the diet or medium. Some investigators favor restricting use of the term to designate conditions where there is a small excess of amino acid causing the relative deficiency; where the excess is large and the disturbance severe they would change the designation to amino acid *toxicity*. There are sound reasons for believing that many so-called amino acid toxicity effects, however, are merely exaggerated imbalance effects. It would seem logical, therefore, to recognize that there are degrees of imbalance and to use a more inclusive definition of the term. It might then be said that effects of amino acid imbalance range from small decreases in rate of growth of animals or micro-organisms to complete failure of growth or continued loss in weight and pathological changes in animals that often lead to fatal termination.

EARLY INVESTIGATIONS

Early studies on the use of gelatin as the source of dietary protein might well be considered as the first studies on amino acid imbalance. Gelatin is not only deficient in tryptophan and methionine but is imbalanced in having a high content of glycine, proline and hydroxyproline. In 1928, Jackson, Sommer, and Rose[1] reviewed the unsuccessful attempts of earlier investigators to use gelatin as a sole source of dietary protein. They then reported data from their own experiments to confirm the fact that gelatin at levels of 15 to 35 per cent was not a suitable source of nitrogen for growth of rats. They stated that this was true even when it was supplemented with the amino acids known to be missing or present in relatively small amounts. They observed deleterious effects at the 35 per cent level as manifested by a high incidence of renal injury and an early fatal outcome of many of the experiments.

In 1944, after the discovery of threonine, Hier, Graham, and Klein[2] again attacked the problem of remedying the amino acid deficiencies of gelatin but without success. They demonstrated the inhibitory effect of glycine, proline, and phenylalanine and attributed the poor growth of rats on gelatin diets to amino acid imbalance.

Another early investigation in which amino acid imbalance was probably involved was that of Curtis and Newburgh.[3,4] They reported that the addition of 0.25 per cent of cystine to an 8 per cent casein diet was harmless but 0.50 per cent was mildly harmful; evidence of injury increased with increasing levels of cystine until at the 5 per cent level the effects were those of a powerful poison. Hemorrhage and necrosis of the liver and hemorrhagic necrosis of the renal parenchyma were present. The production of liver injury by relatively high levels of cystine was confirmed by Sullivan Hess, and Sebrell[5], Lillie[6] and Earle and Victor.[7]

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The complication of most of these early experiments by deficiencies of choline and vitamin E makes interpretation of the results difficult.

The picture was further complicated by the apparently anomalous protective effects of cystine reported by another group of investigators. These workers were engaged in studies of liver necrosis produced in rats primarily as a result of vitamin E-deficient diets. These studies have been reviewed by Schwarz[8] and by Daft.[9] The anomaly seems to have been resolved from the recent discovery[10] that selenium prevents the vitamin E-deficiency liver necrosis; there now is evidence that the protection afforded by cystine is attributable to traces of selenium in cystine.[11]

CONTEMPORARY INVESTIGATIONS

(1) *Effects on Tryptophan Requirements*

In 1945 Elvehjem and associates[12] made the important discovery of the relationship of niacin and tryptophan. Results observed in connection with these experiments led to extensive investigations on amino acid imbalance at the Wisconsin laboratories.[13–15] It was shown that the addition of relatively low levels of gelatin, acid-hydrolyzed protein, or certain amino acids to a niacin-free 9 per cent casein diet markedly reduced the rate of growth of rats. The growth inhibition was prevented by supplementing the diet with niacin. This was the basis of the conclusion that such diet modifications increased the requirement for niacin.

Singal, Sydenstricker, and Littlejohn[16] showed that addition of lysine, valine, histidine, and theonine to a 9 per cent casein, niacin-free diet depressed growth of rats. Niacin or tryptophan prevented the growth depression; tryptophan markedly increased liver storage of niacin. In further studies[17] it was shown that threonine alone, of the four amino acids tested, produced the observed growth depression.

Results reported from our laboratory[18–20] have been in general agreement with those cited above. They have been more definitive, however, in differentiating between tryptophan and niacin requirement effects. As shown by the data in Table I, the growth-depressing effect of adding 2 per cent of acid hydrolyzed casein to a 9 per cent casein diet was prevented by niacin. Even with this small addition of hydrolysate, there was a slight stimulation of growth by supplementation with tryptophan in addition to niacin. When the casein hydrolysate addition was increased to 12 per cent, there was a marked depression of growth despite the presence of niacin. Similar effects were obtained when 10 to 12 per cent of gelatin was added to diets containing marginal levels of tryptophan. The growth depression could be corrected only by tryptophan. The protective action of niacin, when low levels of tryptophan-free materials were added to a 9 per cent casein diet, was apparently related to the tryptophan-sparing effect of the vitamin. Although amino acid imbalance does not appear to affect the basic niacin requirement of the rat, it obviously can affect the amount of tryptophan available for niacin synthesis.

TABLE I

Effect of Tryptophan Imbalance Produced by Casein Hydrolysate Addition to 9% Casein Diet*

Diet supplements			Average gain 4 weeks g/rat	Food/g gain gm
Casein hydrolysate %	Niacin mg/kg	DL-tryptophan %		
—	—	—	21	7.38
—	20	—	73	3.85
2	—	—	14	9.75
2	20	—	90	3.15
2	20	0.05	104	3.08
12	20	—	47	4.62
12	20	0.10	81	3.41

* All diets contained 0.2% choline chloride, 0.30% L-cystine, 1.0% corn oil, complete mineral and vitamin supplements (except niacin, folacin, and vitamin B_{12}) and sucrose to 100%.

(2) *Effects on Requirements for Other Amino Acids*

Harper *et al.*[21–23] have demonstrated a retardation of growth in rats by addition of 1–3 per cent of L-leucine to a 9 per cent casein diet; the retardation was prevented by increasing the casein level to 18 per cent. By using a 9 per cent casein diet supplemented with various levels of a number of amino acids, they have produced growth-inhibiting

imbalance effects between leucine and isoleucine, isoleucine and valine, phenylalanine and isoleucine, and phenylalanine and valine. They also found that supplements of 3 per cent DL-phenylalanine or 3 per cent L-tyrosine depressed the growth rate of rats fed a 9 per cent casein diet supplemented with tryptophan and methionine; this effect was prevented by further supplementation of the diet with threonine.

Sauberlich[24] has reported production of several amino acid imbalances: one caused by addition of 15 per cent hemoglobin to 75 per cent corn diet and corrected by isoleucine; another produced by adding oxidized casein to a peanut meal diet and partially prevented by methionine; a third induced by supplementing 6 to 10 per cent casein diets with essential amino acids less threonine and counteracted by addition of threonine.

Something of the complexity of imbalance effects is indicated by experiments in our laboratory with a basal diet containing 7 per cent of casein and 7 per cent of corn gluten meal (supplemented with 0.30 per cent of L-cystine) as sources of protein. When supplemented with niacin and choline, this diet supports a normal rate of gain of 50 to 60 g in rats in a two-week period. We have previously reported production of a tryptophan deficiency by addition of gelatin to this diet.[19]

The possibility of producing a methionine-deficiency imbalance by the same means was investigated. Since the diet contained sufficient sulfur amino acids for normal growth when supplemented with choline, the choline was omitted in order to increase the total requirement for methionine. The results of this experiment are given in Table II. It was found that 0.24 per cent of supplementary DL-methionine with the basal diet supplemented with 0.20 per cent DL-tryptophan afforded complete protection against kidney damage but did not support normal growth. The further addition of 10 per cent gelatin, however, resulted in 100 per cent kidney damage and 75 per cent mortality. With 0.26 per cent of methionine added to the basal diet or to the basal diet supplemented with either gelatin or tryptophan there was complete protection.

TABLE II

Effect of Supplements With 7% Casein-7% Corn Gluten Meal Diet*

Supplement			Average maximum gain 2 weeks *g/rat*	Kidney damage %	Mortality %
Gelatin %	DL-tryptophan %	DL-methionine %			
—	—	—	14	100	50
10	—	—	15	100	25
—	0.20	0.12	20	75	25
10	0.20	0.12	14	100	75
—	0.20	0.24	34	0	0
10	0.20	0.24	17	100	75
—	—	0.26	38	0	0
10	—	0.26	34	0	0
—	0.20	0.26	38	0	0
10	0.20	0.26	29	100	25
—	0.20	0.36	37	0	0
10	0.20	0.36	51	25	0
10	0.20	0.48	51	0	0
—	—	†	58	0	0

* This diet was supplemented with a complete mineral mixture, thiamine, riboflavin, pyridoxine, calcium pantothenate, niacin, α-tocopherol, calciferol, carotene, 1% corn oil, 0.30% L-cystine and sucrose to 100%. The calculated methionine content of basal diet was 0.43% and tryptophan content 0.12%.

† 0.20% choline chloride added to diet for this group.

But when the basal diet was supplemented with 0.26 per cent methionine and both gelatin and tryptophan, 100 per cent of the rats exhibited kidney damage and the mortality was 25 per cent. With this combination of supplements the protective level of methionine was between 0.36 and 0.48 per cent. An even greater methionine imbalance effect could probably have been produced by omitting cystine as well as choline from the basal diet, but this has not been tested.

We have recently produced a threonine-deficiency imbalance with a slight excess of methionine in an amino acid diet. The diet was constituted principally on the basis of the amino acid content of a 12 per cent casein diet with variations in the content of tryptophan, threonine and methionine. In the experiment reported here, we had expected to obtain evidence of imbalances affecting each of the three amino acids. As shown in Table III, however, the results were clear cut only in that the increased level of methionine depressed growth rate and this was prevented

TABLE III

Effect of Variations in Methionine, Threonine, and Tryptophan in Amino Acid Diet*

Content of three amino acids in diet				
Diet No.	DL-methionine %	DL-threonine %	DL-tryptophan %	Average gain 2 weeks g/rat
S-311	0.20	0.50	0.10	28
S-312	0.20	1.00	0.25	31
S-313	0.40	0.50	0.25	22
S-314	0.40	1.00	0.10	35
S-315	0.40	0.50	0.10	23
S-316	0.40	1.00	0.25	50

* The composition of Diet S-311 was as follows g/kg L-arginine HCl 4.0, L-histidine HCl 4.0, L-lysine, HCl 7.5, L-tyrosine 4.0, DL-tryptophan 1.0, DL-phenylalanine 6.0, L-cystine 2.0, DL-methionine 2.0, DL-threonine 5.0, L-leucine 10.0, DL-isoleucine 10.0, DL-valine 14.0, L-glutamic acid 20.0, L-aspartic acid 3.0, glycine 2.0, DL-alanine 3.0, L-proline 2.5, DL-serine 2.5, L-asparagine 3.0, salts No. 6 50, $NaHCO_3$ 6.5, choline Cl 3.0, Cod liver oil 10, corn starch 250, cellulose 10.0, sucrose 415, lard 150; (mg/kg penicillin G (Na) 100, folacin 2, menadione 5, riboflavin 4, thiamine 4, pyridoxine 4, Ca pantothenate 10, niacin 25, inositol 200, biotin 0.5, α-tocopherol acetate 120). The other diets were identical except for variations in the methionine, threonine, and tryptophan, offset by corresponding variations in sucrose.

by increased threonine. The growth depression was no greater when both methionine and tryptophan were increased, than when only methionine was increased. When both methionine and threonine were increased, there was no inhibition of growth as compared with the basal levels, although growth was considerably less than when all three amino acids were increased. A competitive antagonism between methionine and threonine has been reported by Teas, Horowitz, and Fling[25] and by Doudney and Wagner.[26] Pending further study, we are inclined to regard the antagonism observed in our study as specific for the particular diet combination rather than an indication of a specific antagonism of methionine for threonine.

(3) *Effects on Liver Lipid Values*

In 1953 Singal and associates[27,28] reported the production of fatty livers in rats receiving adequate dietary choline. The condition resulted when rats were restricted to low level casein diets or to amino acid diets containing suboptimal levels of threonine. Increasing the threonine level in either diet reduced liver lipid to normal values.

Harper *et al.*[29,30] also observed the lipotropic action of threonine as a supplement to low-level casein diets. Sauberlich[31] has produced fatty livers in rats by feeding diets containing, as the sole source of protein, corn, sesame meal, wheat gluten, or 6–10 per cent of casein. Individual supplements of cystine, methionine, lysine, histidine, threonine, phenylalanine, tryptophan, glycine or glutamic acid usually improved growth but increased liver lipid. Supplementing a 7 per cent casein diet with 10 per cent of zein, lactalbumen or casein or with adequate levels of ten essential amino acids resulted in normal liver lipid values. This suggests that production of fatty livers by certain low-protein diets supplemented with choline is a result of amino acid imbalance in which various amino acids may be involved. Threonine may have a lipotropic action only under conditions where there is a negative threonine imbalance. It is readily conceivable that under certain conditions threonine might even be antilipotropic.

It is well known that, when rats are fed diets containing 32 to 34 per cent casein or 18 to 20 per cent casein supplemented with methionine or betaine, they can synthesize sufficient choline to meet their requirements. Some surprising results were obtained with a 60 per cent casein diet in our laboratory. As shown in Table IV, when choline was omitted from this diet, depression of weight gains, development of moderately fatty livers, and considerable kidney injury were noted.

Methionine or betaine equivalent to 0.20 per cent choline aggravated the condition. Aminoethanol was as effective as choline. The results suggest an imbalance involving an excess of methionine and either a deficiency of aminoethanol precursors or an inhibition of the production of aminoethanol from precursors. Studies now in progress indicate that neither glycine nor serine is as effective as aminoethanol for supplementation of the 60 per cent casein diet.

TABLE IV

Effect of Various Supplements With 60% Casein Diet on Weight Gain, Kidney Damage, and Liver Fat of Rats*

Supplement %	Average gain 2 weeks g/rat	Kidney damage %	Liver fat† %	Kidney fat† %
Choline Cl 0.20	56	0	—	—
None	43	75	25.7	17.8
Methionine 0.64	34	75	27.2	17.5
Betaine HCl 0.66	30	25	31.1	20.6
Aminoethanol 0.09	62	0	10.0	12.2
Methionine 0.64 and aminoethanol 0.09	52	0	11.1	11.5

* All diets contained 60% casein, complete mineral and vitamin supplements, including vitamin B_{12} and folacin, 10% corn oil and sucrose to 100%. Choline was omitted except where indicated.

† Dry weight basis.

(4) *Relation to Amino Acid Toxicity*

The toxic effects of various amino acids administered at relatively high levels under various conditions have been demonstrated by a number of investigators. I have already mentioned some of the early work on cystine. Allison and co-workers[32,33] have reported that rats, fed a 12 per cent casein diet to which 4.8 per cent DL-methionine was added, lost weight rapidly over a 20-day period. The weight loss was decreased by adding 1.7 per cent L-arginine and 4.8 per cent glycine to the methionine supplemented diet. Hardin and Hove[34] found that increasing DL-methionine from 0.5 per cent to 2.5 per cent in a 10 per cent casein diet decreased growth (four weeks) by 69 per cent in the absence of vitamin E, folacin, and vitamin B_{12} but only by 39 per cent in the presence of these supplements. With further supplementation of the diet with arginine and lysine in molecular equivalents to the 2 per cent increase in methionine, the repression of growth was only 5 per cent. Russell *et al.*[35] determined the effect on growth of rats from addition of excess of each of the ten essential amino acids to the diet. They used a 10 per cent casein diet supplemented with amino acids to the levels suggested by Rose and Womack.[36] Then each essential amino acid was added to a separate test diet to supply the active isomer in 200 per cent excess of the standard level. Only DL-lysine and DL-methionine reduced the average grain significantly below that of the rats on the standard diet. They concluded that the growth repression was a property of the two compounds and was not due to amino acid imbalance.

Some interesting studies on toxicities of essential amino acids administered by intraperitoneal injection have been reported from Greenstein's laboratory.[37] When administered individually, L-tryptophan, the most toxic, was 6.5 times as toxic as L-isoleucine, the least toxic of the L-isomers. Interestingly when the amino acids were administered in a mixture, proportioned in accordance with their individual toxicities, the toxicity of the mixture was much less than that calculated from the mean of the toxicities of the individual components. This was attributed mainly to the effect of arginine in the mixture, although when administered separately, L-arginine ranked next to L-tryptophan in toxicity.

We are presently studying effects of adding relatively large amounts of individual amino acids to the basal diet we have used as a standard in our chronic choline deficiency investigations. This diet contains 6 per cent casein, 25 per cent peanut meal, 1 per cent cod liver oil and 19 per cent lard with complete mineral and vitamin supplementation, except for vitamin B_{12} which was used only in certain treatments. For the amino acid experiments, the diet was supplemented with 0.30 per cent of choline chloride. Methionine was added at a level of 4 per cent in the diet and the other amino acids were added at molecular equivalent levels.

The effects of these additions on growth of rats are shown in Table V. Of the 12 amino acids tested with this diet, methionine exhibited by far the greatest repression of growth. L-Cystine ranked next but was substantially less inhibitory than methionine. The harmful effect of methionine was markedly lessened by

TABLE V

Effects of Excess Amino Acids Added to 6% Casein, 25% Peanut Meal, 20% Fat Diet

Amino acid	Level in diet %	Average gain 4 weeks g/rat
Basal diet only	—	125
L-methionine	4.0	24
DL-methionine	4.0	23
L-cystine	6.4	62
DL-methionine	4.0	
Gelatin	12.0	70
DL-tryptophan	0.15	
DL-tryptophan	5.5	80
L-lysine·HCl	4.9	82
L-histidine·HCl	5.6	86
L-arginine·HCl	5.6	91
L-leucine	3.5	97
Glycine	2.0	111
DL-threonine	3.2	115
DL-phenylalanine	4.4	115
DL-valine	3.1	118
DL-isoleucine	3.5	119

12 per cent of gelatin. This protein has been used so much for the production of imbalance effects, it is interesting to find a situation in which it partially corrects a severe imbalance. This result at least suggests that the so-called toxicity of methionine is really an amino acid imbalance effect. It may be possible to correct it completely by restoration of the amino acid balance.

DL-Tryptophan, L-lysine, L-histidine, L-arginine, and L-leucine were much less harmful than methionine but were still significantly depressing. Glycine, DL-threonine, DL-phenylalanine, DL-valine, and DL-isoleucine had only slight effects. It remains to be determined whether the L-isomers of the last four amino acids have any greater effects than the DL-mixtures.

It should be remembered that the basal diet used in these tests contained slightly more than 20 per cent of protein. It supported an excellent rate of growth. It would not be surprising to find more severe effects, or differences in the order of effects, if similar amounts of amino acids were added to lower protein diets.

SIGNIFICANCE IN NUTRITION

The basic cause of amino acid imbalance effects has not been completely explained. Specific antagonisms between or among certain amino acids have been postulated and have received support from work on *Neurospora* mutants. Relationships in molecular size and structure, in utilization or disposal pathways, or in positions in the peptide chains might favor such antagonisms. If they exist in animal nutrition, they may be more of a quantitative than of a qualitative order. It is not yet clear whether such antagonisms as have apparently been indicated by rat growth experiments are specific for the amino acids involved, or merely for the quantitative relationships of the amino acid moiety of the diet treatment used. Further studies on this point are needed.

At the present time no one seems to have proposed a more plausible hypothesis of amino acid imbalance effects in animals than the one originally suggested by the author in 1954.[19] This is that when a single amino acid is inadequate in amount for maximum production of tissue proteins, any surplus of other amino acids added must be excreted or disposed of in some way; in the process there is a wasting of the limiting essential amino acid, which increases the severity of the deficiency. Supporting this hypothesis are data that show a greater excretion of amino acids from incomplete or imbalanced proteins than from complete proteins as reported by Sauberlich *et al.*[19,38,39] and Schweigert.[40] The excretion of unchanged amino acids, however, may not account for all of the imbalance effects. Part of the surplus may be excreted in the form of metabolites.

The probable relationship of amino acid imbalance to toxicity effects certainly is worthy of further investigation. Studies to determine whether long term imbalances of moderate severity would produce pathologic changes in animals might also be rewarding.

Information now available indicates some of the difficulties in determining amino acid requirement levels for general application even within a given species. The levels of nitrogen and energy intake and the balance of amino acids from which the dietary nitrogen is derived markedly affect requirements. Certainly

a comprehensive understanding of amino acid balance offers possibilities for more efficient utilization of these nutrients. Such knowledge is an essential prerequisite to any program of amino acid fortification of dietary proteins. Research in this field may make possible the eventual downward revision of present recommendations on requirement levels of dietary protein or amino acids.

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Protein Metabolism in Chronic Infantile Malnutrition (Kwashiorkor)

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MALNUTRITION may be considered the world's foremost pediatric problem of our time. It is particularly prevalent in technically underdeveloped countries where, directly or indirectly, it is a major contribution to child mortality.

Regardless of its specific cause, the functional lesions of the syndrome conform to a quite constant pattern. The clinical picture however, differs according to a variety of circumstances such as: severity of the illness, chronicity, color of the skin, color of the hair, age of the patient, degree of exposure to sunlight, climate, etc.

Among cases of chronic severe infantile malnutrition, two main clinical pictures may be identified: one, the type variously known as "kwashiorkor" "Mehlnährschaden," "distrofia-da farine" etc.; the other, the "marasmic" or "atrophic" baby. However, most of the cases belong to intermediate types, sometimes referred to as "marasmic kwashiorkor."

In our series, children of the first group have a mean age of 38 ± 18 months, contrasting with the younger age of the "atrophic" group, who on an average were 23 ± 15 months old. Clinical records show a history of over-all nutritional deficiency since the age of weaning, calorie intake being somewhat less precarious than the remainder of the nutritional complex. The situation has become aggravated in a period of varying lengths of time previous to hospitalization, during which the child has suffered either from an acute infectious disease or from repeated bouts of diarrhea and vomiting. Upon admission to the hospital there is generally pitting edema and gross muscular wasting, in spite of which, considerable amounts of subcutaneous fat can still be found on several portions of the body. If the child survives, these may later disappear and the child gradually resembles more and more a case of "marasmus." Many of the marasmic babies, however, have never shown pitting edema, but do give a history of striking nutritional deficiency since their very first days of life; frequently, no history of recent acute episodes can be ascertained.

As suggested by Waterlow,[1] and in order to avoid discussion about nomenclature, the incidence of the main clinical lesions as shown by either clinical type is presented in Table I.

Data obtained so far have shown that despite the difference in clinical appearance, both groups are comparable with regard to essential features, such as general overhydration, including chemical edema in the skin. Distribution of water compartments seems to differ a little, in the sense that there is a greater tendency for cases of "kwashiorkor" to show intracellular overhydration and decreased potassium concentration.[2]

This separation of marasmus and kwashiorkor, although most interesting from a descriptive viewpoint, may be of little practical importance since both types have the same prognosis, respond equally well to the same treatment and recover in a strikingly similar pattern.[3,4]

At the individual level, chronic severe

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TABLE I

Clinical Lesions Found on Admission in 584 Children with Severe Malnutrition

Lesion	Without pitting edema %	With pitting edema %
Dry hyperchromic skin	90	94
Very dry hyperchromic skin with mosaic appearance	38	46
Follicular hyperkeratosis	20	26
Hyperkeratosis palmaris et plantaris	48	37
Fissures	3	8
Seborrhea	42	49
Pellagrous erythema	13	47
Acute pellagrous dermatitis	18	40
Dyskeratotic hyperchromic lesions	50	83
Desquamating lesions in large flaps	8	21
Desquamating lesions in small flaps	13	37
Postdesquamation hypochromia	15	26
Hyperchromia along capillary circulation	1.4	8
Crusty lesions suggesting postpuric lesions	6	11
Purpuric lesions	24	28
Perifolliculitis	1.4	11
Coldness and cyanosis of hands and feet	62	82
Marbleization	11	18
Telangiectasis	1.4	6
Gangrenous lesions and eschars	11	18

malnutrition is the result of a poor diet, eaten in amounts that fall well below the minimal requirements for the age. Foodstuffs of animal origin are very seldom consumed. The total protein intake is often no more than 60 per cent of the recommended allowance.[5]

Infections, especially those that are accompanied by diarrhea, may be common, and during these frequent episodes the nutrient intake practically drops to zero with the exception of some calories derived from the sugar used to sweeten various infusions which alone, or together with some carbonated beverages constitute the total ingestion during periods that may last from one to several days.

Since proteins of animal origin are ingested in such low amounts as to be considered a luxury by many of our patients, our attention has been focused to the study of protein metabolism in these cases.

Serum Proteins: Regardless of the analytical method employed, the results show a similar

TABLE II

Serum Proteins in 395 Cases of Chronic Infantile Malnutrition

	Children with clinical edema			Children without clinical edema		
	M	S.D.	S.E.	M	S.D.	S.E.
Total proteins	4.36	1.03	0.05	5.63	0.90	0.10
Albumin	1.64	0.70	0.04	2.59	0.70	0.08
Globulin	2.71	0.70	0.04	3.02	0.99	0.11
Alpha-globulin	0.71	0.38	0.02	0.83	0.31	0.04
Beta-globulin	0.80	0.50	0.03	0.86	0.42	0.05
Gamma-globulin	1.25	0.47	0.03	1.32	0.45	0.05

M = mean; S.D. = standard deviation; S.E. = standard error.

picture as illustrated by Table II which presents the average values in 393 cases. The pattern could be described as dysproteinemia with hypoalbuminemia and hypergammaglobulinemia. The concentration of total protein is decreased in most cases regardless of the presence or absence of pitting edema, but with the exception of gamma globulin, the figures tend to be higher in "marasmus" than in "kwashiorkor." There is an inverse relationship between albumin and alpha-globulin concentrations, with a higher significant correlation for the group of edematous cases ($p < 0.01$).[6]

Since the plasma albumin concentration represents the result of a balance between synthesis, distribution in the body and degradation, any or all of these factors could explain the low values found. Their relative influence has been evaluated through isotope studies.

I^{131}-labeled human serum albumin was given intravenously, to 14 severely malnourished children immediately after their admittance to our department; in eight of them a second test was done when they were well in the stage of recovery. The specific activities of the labeled albumin were such that no more than 1 mg of iodinated albumin was injected, per child; this amount is equivalent to less than 1.5 μc/kg/body weight. The disappearance of radioactivity from the serum was followed for about 27 days.

It has been found that the average half-life of albumin is 9.3 days (range 6.6 to 11.5 days) during the first period and 9.8 days (range 7.0 to 11.3) for the second study. There is no significant difference between these sets of values, nor could a real difference be found in the vascular-extravascular distribution of albumin in one period as compared with the next. None of the radioactivity found in the urine was bound to protein, nor was there demonstrable proteinuria.

The individual half-lives of albumin bear no relation to the type of diet administered nor to the type of malnutrition, and they are independent of the changes in the concentration of plasma albumin.

The results may be interpreted as an indication that, unlike other species, the human organism is unable to compensate for an excessively low-protein intake. The rate of catabolism for animals suffering from protein deprivation can be reduced. Humans on the other hand, maintain their rate of catabolism. Thus, the primary defect in our malnourished children appeared to be a lack of synthesis due to a quantitative deficiency of precursors.[7]

TABLE III

Mean Daily Intake of Essential Amino Acids
(Protein intake 36.71 g/body weight/10 kg)
Whole milk = 1,224 ml
Beans puree = 360 g = 98.3 g raw beans
Corn meal = 240 g

	Corn and beans *g*	Cow's milk *g*	Requirements (L. E. Holt Jr.) *g*
Tryptophan	0.36	0.367	0.30
Valine	2.55	1.74	—
Threonine	1.91	1.20	0.60
Methionine	0.64	0.46	0.65
Lysine	1.89	2.35	0.96
Histidine	1.06	0.92	—
Leucine	5.87	2.85	—
Isoleucine	3.63	1.56	0.90
Phenylalanine	2.69	1.10	0.90
Arginine	1.77	0.83	—

Since the chief, and many times the only, constituents of the diet, of a large percentage of the malnourished population of Mexico, Central America, South America, and of several parts of Africa and Europe are corn and beans, and since recovery in some severely malnourished children can be initiated with no other treatment than a diet of corn meal and beans, the magnitudes of the nitrogen absorption and retention of this poor diet have been compared with those of cow's milk.

In Table III the amino acid content of a 4:5 mixture of corn and beans as determined by a microbiologic method is compared with that of cow's milk.

It may be seen from Table III that, with the exception of lysine, the amino acid content of corn meal and beans, when mixed in the stated portions, is equal to or greater than the content provided by a similar weight of cow's milk protein and well above the requirement of essential amino acids for growth in normal infants.[8]

Balance data on 17 children, all showing the typical signs and symptoms of severe malnutrition are presented in Tables IV and V.

TABLE IV

Mean Daily Calorie Intake, and Intake, Excretion, and Balance of Nitrogen by Severely Malnourished Children on a Diet of Corn and Beans[9]

Child	Calorie intake *cal*	Intake *g*	Urinary *g*	Excretion fecal *g*	Total *g*	Balance *g*	Retained (*mg/kg/* body weight)	Intake (*mg/kg/* body weight)
1 O. R. G.	996	5.34	0.45	5.04	5.49	−0.15	−17	600
2 R. L. R.	500	3.26	0.46	1.92	2.38	+0.88	+135	504
3 J. C. F.	1,162	8.50	0.93	4.48	5.41	+3.09	+274	752
4 H. V. H.	332	2.81	0.77	1.68	2.45	+0.36	+40	313
5 A. R. G.	336	2.15	0.87	1.85	2.72	−0.57	−72	269
6 R. H. M.	340	1.54	0.84	1.24	2.08	−0.54	−111	316
7 L. L. L.	337	2.29	0.62	0.97	1.59	+0.70	+102	333
8 C. T. C.	429	2.00	0.77	1.91	2.68	−0.68	−85	252

TABLE V

Mean Daily Calorie Intake, and Intake, Excretion, and Balance of Nitrogen by Severely Malnourished Children on a Diet of Cow's Milk[10]

Child	Calorie intake kg/day	Intake mg N/kg/day	Urinary excretion mg N/kg/day	Absorption		Retention		
				mg/N/kg/day	Intake %	mg N/kg/day	Intake %	Absorption %
1	64	395	152	341	86	185	47	55
2	60	459	156	394	86	235	51	60
3	76	448	148	390	87	239	53	62
4	94	466	180	332	71	148	52	46
5	50	310	94	184	59	83	27	46
6	139	653	114	477	73	409	63	85
7	136	576	222	531	92	310	54	58
8	60	338	112	276	82	160	47	54
9	82	401	145	237	59	92	23	39

Eight subjects received a corn and beans diet, while the other nine were fed cow's milk.

In efforts to cancel differences in intake due to different age, weight, and appetite results have been expressed in terms of percentage of nitrogen intake, Table VI.

TABLE VI

Nitrogen Absorbed and Nitrogen Retained by Severely Malnourished Children, on Diets Either of Corn and Beans or Cow's Milk, Expressed as a Per Cent of Nitrogen Intake

Child	N absorption as a per cent of N intake	N retention as a per cent of N intake
Diet: corn and beans		
1	5.6	− 2.8
2	41.1	26.9
3	47.3	36.4
4	40.2	12.82
5	13.8	−26.7
6	19.2	−35.3
7	57.6	30.6
8	4.8	−33.6
Diet: cow's milk		
1	91	47
2	86	51
3	86	53
4	72	32
5	58	27
6	73	63
7	92	54
8	82	47
9	59	23

It is apparent that nitrogen absorption and nitrogen retention are extremely variable in the children fed on corn and beans. Four children are in positive nitrogen balance while in four the balance is negative. No correlation has been found between the calorie intake and the nature of the balance, nor with any specific clinical sign or syndrome.

On the other hand, the figures of nitrogen absorbed and retained by the malnourished children fed on cow's milk are equal or greater than those found in well-nourished children of a similar age.

Regression lines for the relationship between intake and absorption or retention show that the magnitude of the slope is greater for cow's milk, and the difference from the corresponding slope obtained for the corn and bean's diet is highly significant ($p < 0.001$) (Figures 1 and 2).

These results support the concept that the biologic value of a protein depends not only on its amino acid content, and that other

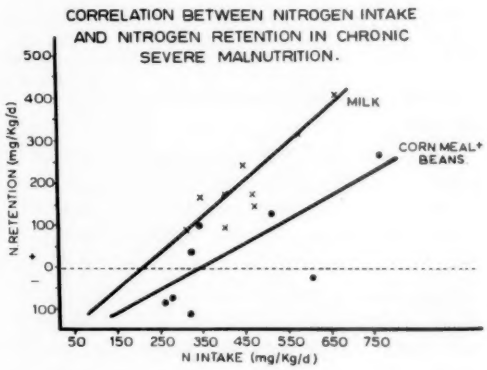

Fig. 1.

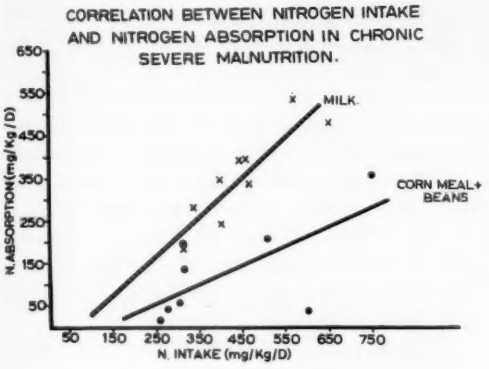

Fig. 2.

factors, such as the relative proportions of some amino acids, must play a role.

It may be pointed out that animals fed on a diet of corn and beans thrive poorly as compared with animals fed on milk proteins,[11] but the fact that 50 per cent of the children who received the corn and beans diet showed positive nitrogen balances, may help to explain why some children develop severe protein malnutrition while others fail to do so in the presence of the same basic dietary pattern.

As stated before, malnourished children are often afflicted with bouts of diarrhea primarily attributable to poor sanitary conditions, the attacks occurring in increasing severity. The possibility that during these episodes of diarrhea, nitrogen absorption or intestinal excretion could be altered in such a way as to be a real contribution to protein depletion has been entertained.[12] Results of balance studies designed in this direction have been expressed in terms of the weight of the fresh feces rather than the number of stools, and relate the weight of fresh feces excreted per day to the absorption taken as a per cent of the corresponding intake.

In order to cancel individual differences, balance results have been divided into two groups. The first one comprises the data found in children who "excreted" less than 100 g of fresh feces per day. The second group has the values obtained when the fresh feces weighed more than 400 g/day. During the balance periods, whole cow's milk with 10 per cent added carbohydrate was given as the only food. There was free intake of water. Electrolyte solutions were administered, orally or intravenously, when necessary. The mean values for 26 children with a total of 48 balance periods are entered in Table VII.

Analysis of variance shows that the linearity of the relation between intake and absorption does not change significantly when more feces are excreted per day (Figures 3 and 4).

TABLE VII

Influence of Diarrhea on the Nitrogen Absorption Measured as a Per Cent of Nitrogen Intake in Children Afflicted with Chronic Severe Malnutrition

	N absorption as a per cent of N intake		
	Average	Standard deviation	Standard error
Children with excretion of less than 100 g of "fresh feces" per day	82	14	2.6
Children with excretion of more than 400 g of "fresh feces" per day	75	8	2

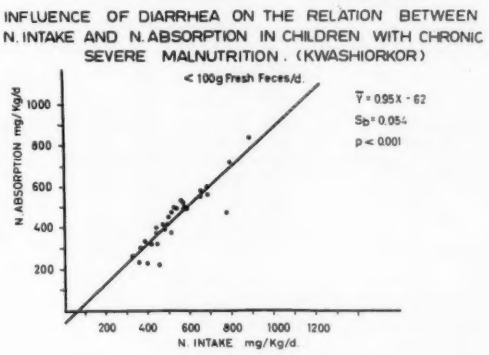

Fig. 3.

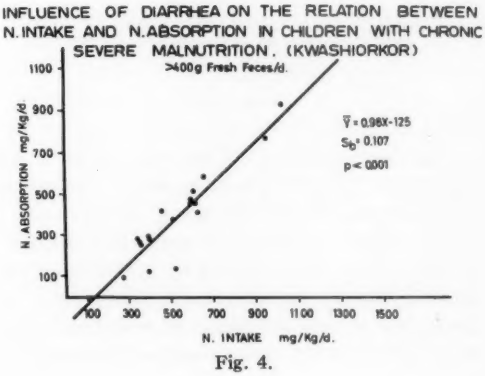

Fig. 4.

This phenomenon, already reported in well-nourished babies suffering from diarrhea[13] reveals that the prevalent practice of withholding food during diarrhea can be dangerous, especially in malnourished subjects, who, because of the decreased percentage of absorption need now a larger intake in order to keep the same absolute figures for absorption and consequently for retention.

Figures 5 and 6 show that diarrhea does not appreciably influence the relation between

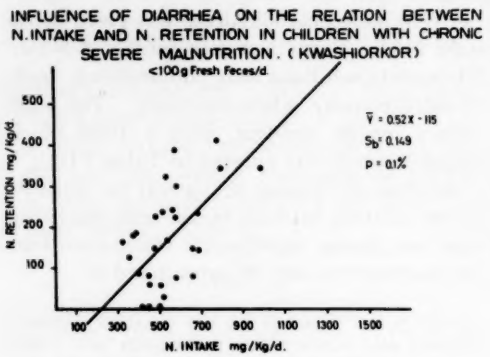

Fig. 5.

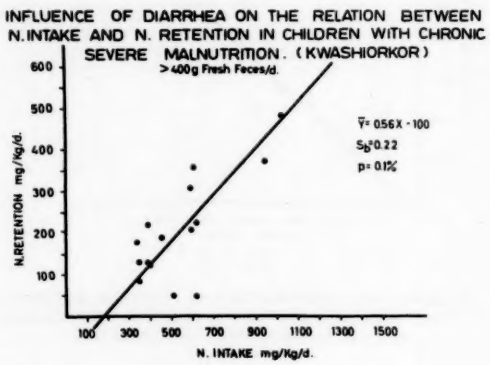

Fig. 6.

intake and retention. Similarly, there is no significant change in the nitrogen retained when this is expressed as a per cent of intake.

Our opinion favors the theory that diarrhea plays a minor role in regard to modifications of protein absorption and excretion. Thus it seems that it is the common practice of reducing the food intake to a minimum which causes a more or less acute lack of precursors for protein synthesis in an already protein-depleted organism.

In coming to the conclusion that the dietary proteins available for consumption by a large percentage of the world population are poorly absorbed and poorly retained, and considering that food habits, once established, are extremely difficult to be changed, it became necessary to test whether the addition of supplements could improve the biologic value of such diets.

A preliminary experience has been published.[14] It showed that when corn meal and beans are supplemented with L-lysine and L-tryptophan, in doses of 30 to 40 mg of amino acid/kg/body weight/day, both absorption and retention expressed as a per cent of intake, were greater than in the nonsupplemented period. In the series of four children studied, one changed from a negative to a positive balance after the ingestion of the amino acids.

Since at times one may have a limited and insufficient intake of good quality proteins, such as cow's milk proteins, and since there have been reports on the beneficial influence of lysine added to milk,[15] one might think that this supplementation would be a better way of making use of an expensive food. Although Holt[16] has called attention to the fact that cow's milk covers the lysine requirements of the growing infant quiet well, we do not know the requirements of malnourished children. Therefore, the effect of lysine on the utilization of nitrogen was studied.[17]

Five children were fed milk with and without the supplement. The modifications of the appetite were in all cases similar to the general sequence recorded in children without supplementation of any kind. This increased interest in food is one of the most constant phenomena observed during the course of recovery from malnutrition and it is used as a sign of a good prognosis.

As seen in Figures 7 and 8, lysine supplements had practically no significant effect upon absorption. In regard to retention there was no significant effect in three cases; while in the other two children (R. R. J. and C. E. A.), retention increased in comparison to the

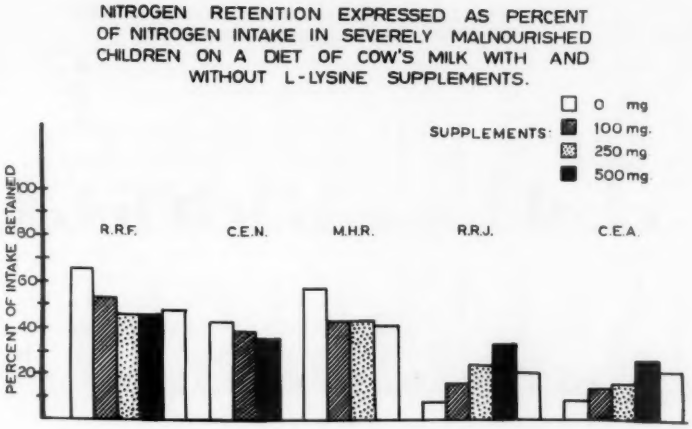

Fig. 7.

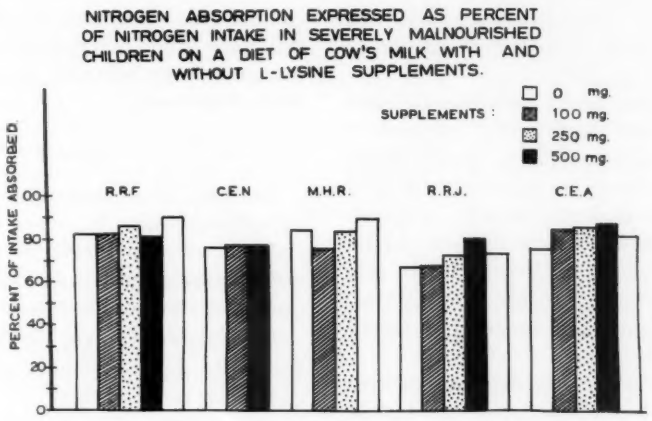

Fig. 8.

figures obtained during the first control period, but the increment has no statistical significance when tested against the second control period obtained at the end of the study.

Flodin[18] has called our attention to the fact that the supplements of lysine seem to be effective only in children who fail to show good nitrogen retention. The data from child R. R. J. and the importance of proving a beneficial effect, even if only on the very few children with chronic severe malnutrition who fail to show good retention, seems to warrant extending our observations to a large number of subjects.

It has been suggested[18] that within the picture of chronic severe malnutrition there might be one or more specific amino acid deficiencies, which could be responsible for some clinical and biochemical findings. With these ideas the group of Holt at Bellevue Medical Center in New York, and our group in Mexico have started the exploration of amino acid metabolism in this disease.

Amino acid partition in urine revealed a low threonine output. Isoleucine was excreted in higher amounts and proportions than leucine, and phenylalanine figures also were higher than those of tyrosine. Amino aciduria was present during recovery.[19]

The levels of free amino acids on blood plasma have been quantitated by a chromatographic method using columns of Dowex

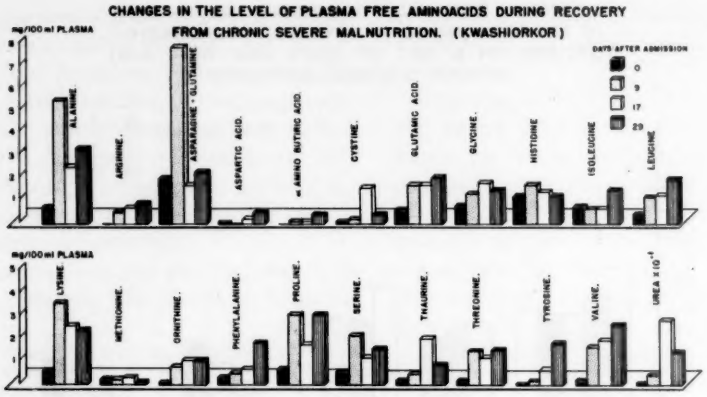

Fig. 9.

50-X5. Figure 9 presents the changes observed in the amino acid concentrations of a child with all the classic signs of protein malnutrition of the "kwashiorkor" type.[20]

The first sample, taken immediately after admission to our department, showed a pronounced hypoaminoacidemia, with a figure for total free amino acids of about one-third of the value found in normal infants. Glycine, histidine, and isoleucine were the only amino acids not reduced. The lowest levels corresponded to tyrosine, valine and cystine, which were of the order of one-tenth or less of the normal minimal.

With the exception of phenylalanine, cystine, and tyrosine, the levels found after nine days of treatment were normal, tyrosine still giving the lowest figure, and it was not until the last specimen taken on the 29th day after admission that this amino acid showed a normal value.

Very low values of tyrosine have been obtained in six other typical cases studied in Mexico, and Holt has noted this same finding in the blood of three other severely malnourished children, one from French West Africa, and the other two from Nigeria.

In 1955 on the basis of the urinary excretion data, Holt *et al.* suggested a deficiency of the enzyme which converts phenylalanine to tyrosine. The findings in blood plasma seem to support this hypothesis. Its confirmation by direct assays of phenylalanine hydroxylase would reveal one more instance where chronic severe malnutrition produces, among its clinical or biochemical characteristics, a syndrome which differs from a well-established disease only in being reversible with an adequate treatment.

SUMMARY

In trying to explain the causes for plasma hypoalbuminemia in severely malnourished children, increased rate of degradation and disturbances of protein absorption or retention can reasonably be eliminated.

The deficient protein synthesis seems to be due to the low ingestion of a poorly absorbed and poorly retained diet.

"Diarrhea" causes in some children diminution of the nitrogen absorption, but it appears that it is the common practice of reducing the food intake to a minimum during the bouts of diarrhea which lead to an acute lack of precursors superimposed on an already chronically depleted organism.

Preliminary experiences show that supplements of lysine and tryptophan improve the biologic value of the corn and bean diet. No beneficial effect on the absorption or retention of nitrogen could be demonstrated when cow's milk was supplemented with L-lysine.

Data on free amino acids of blood plasma seem to support the hypothesis that phenylalanine hydroxylase is deficient in children with chronic severe malnutrition of the kwashiorkor type.

ADDENDUM

Since the time of the presentation of this paper we have studied the free amino acids in blood plasma of 13 severely malnourished children. Our main findings are as follow:

(1) All but two of the children, one of the kwashiorkor type and one belonging to the marasmic type, showed a decrease level for total amino acids.

(2) Arginine, leucine, and valine were the only amino acids found below normal levels in all 13 patients.

(3) No correlation was found between the total amino acid concentration and the clinical type of malnutrition, its apparent duration, or the estimated degree of protein depletion as judged by the plasma-albumin figures.

(4) Twelve out of the 13 children had an abnormal ratio of phenylalanine/tyrosine.

(5) Neither the clinical type of malnutrition, nor its severity, chronicity or the estimated degree of protein depletion could be correlated with the values of the ratio phenylalanine/tyrosine.

It is our opinion that the findings are compatible with the view that these disturbances of the metabolism of aromatic amino acids may represent a biochemical sign of arrested growth and development.

Studies on the activity of the system phenylalanine-hydroxylase in nomal and malnourished children are under way at our laboratory and a detailed report of these 13 cases is now in preparation.

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Reflections Upon Some Lipotropic Facts and Fantasies

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THE OPPORTUNITY to be here today to join with others in paying respect to Dr. Sydenstricker gives me great pleasure. As the only Canadian on the program I bring greetings and best wishes to Dr. Sydenstricker from his many admirers in Canada. Few Canadians have had the good fortune to know Dr. Sydenstricker personally, but his work in human nutrition and hematology is, of course, well known to many of us. Professor Best is sorry that he cannot be present today. He has asked me to convey his personal congratulations and best wishes to his good friend on this happy occasion.

When Dr. Singal wrote asking me to take part in today's program I was highly honored. Later, as I realized that I was expected to discuss lipotropic phenomena at a Symposium dealing with problems of human nutrition my euphoria subsided. Cogitating upon the fact that while a great deal is known about lipotropic phenomena in rats and somewhat less in other animals, and that relatively little accurate information is available concerning the role of choline and its dietary precursors in man, the word "fantasy" flitted through my mind. It suggests a supposition based on no solid grounds, which may seem appropriate to a discussion of lipotropic phenomena in man. However, the dictionary reminded me that while the commonly accepted meaning is one of fanciful or delusive imagination, the original Greek root (*φαντασια*) meant literally "a making visible" and was derived from the verb meaning "to show." Thus I was happily left free by the title assigned to me to show you (if possible) how lipotropic agents act in clinical situations, or if I could not convince myself or my audience that there is a definite effect I could leave the matter of lipotropism in man up in the air as being something between a supposition without much solid foundation and a whimsical delusion. Possibly I should begin by reviewing briefly some of the facts upon which most workers seem to be in agreement.

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CHOLINE

Choline is a quaternary ammonium base that fulfills some of the requirements of a vitamin, being an organic compound necessary for normal growth, which is not used to supply energy. However, because it is used as a constituent of a building unit of the body and because the amounts required are measured in milligrams rather than micrograms many nutritionists decline to accept it as a vitamin. Whether one chooses to call choline a vitamin or merely a protective substance, it must be accepted as an important accessory food factor since in its absence growth fails and characteristic lesions develop in species as varied as dogs and ducklings or monkeys and mosquitoes.

Fatty livers due to lack of choline have been observed in at least 10 species of animals: rats,[1,2] mice,[3,4] dogs,[3,5,6,7,8] rabbits,[9,10] hamsters,[11] guinea-pigs,[12,13,14] calves,[15] pigs,[16,17] monkeys,[18,19] and ducklings.[20] In some species (e.g., the dog and rat) the liver is easily made very fatty, the deposition of glycerides sometimes reaching excessive amounts, (from 20 to 40 per cent of fresh liver weight). In

other species (viz., the rabbit and especially the guinea-pig) it is more difficult to produce fatty livers of this type. The difficulty is apparently due to the dislike of these animals for the unnatural, choline-deficient rations. Mulford and Griffith[21] showed that when the intake of food is small, even rats fail to develop fatty livers on choline-poor diets that produce a great deposition of fat when the same food is consumed more liberally. The total calorie intake must be in excess of a certain minimum before fat will accumulate in the liver.

It should always be kept in mind that fatty livers may arise from other causes—infections, toxic chemicals, hepatic anoxia, or other deficiencies that interfere with the function of the hepatic cells. It is sometimes said that these fatty livers do not respond to treatment with choline. It is more correct to say that the presence of choline in the diet fails to prevent the development of the lesion. Such fatty livers cannot be cured in the absence of choline from the diet.

The pathologist may sometimes see considerable stainable fat in liver sections before there is any notable increase in the amount of total lipid material extractable from the tissue by hot alcohol or other suitable solvents. This latter condition is observed, for example, when rats are fed diets containing choline but that are low in protein.[22,23a,b]

There is a certain amount of confusion as to the severity of fatty livers obtained under different dietary conditions, in different laboratories. Some workers express their results on a fresh-tissue basis,[2,4,8,22] others refer the data to a fat-free basis,[24] others again prefer to use the dry weight of the tissue as the basis[25a,b] and some use the fat-free, dry weight.[26] The average values for normal liver lipids in young adult male rats of our colony, expressed in the four ways are 6.3, 6.7, 22.7 and 29 per cent, respectively, and a moderately fatty liver gave values of 15, 18, 42 and 75 per cent, respectively, when calculated to the different bases of reference.

Cirrhosis has been observed in rats[27] and dogs[8] maintained for long periods on choline-deficient diets. Chronic choline deficiency in the rat leads to the development of various types of neoplasms[28,29,30] in a considerable number of animals. Cirrhosis has also been reported in mice[31] and rabbits,[32] but in our laboratory only minimal fibrosis was obtained under these dietary conditions in mice, with a large proportion of hepatomas developing without cirrhosis.[33]

Choline deficiency leads to a number of other abnormalities. The hemorrhagic-kidney syndrome, so easily obtained in young rats,[34] cannot be produced in young mice or puppies, although both develop fatty livers readily. Young pigs[17] and calves[15] develop a renal lesion that resembles that seen in rats; rabbits develop a nonhemorrhagic renal lesion that can be prevented by choline.[35] Hemorrhagic lesions have been described in the heart muscle and adrenals of rats fed choline-deficient diets.[36] Involution of the thymus has been noted.[34] Muscular weakness has been reported in both rats[37] and pigs,[17] and muscular dystrophy in rabbits[38] fed choline-poor diets. Bradycardia[39] has been observed in choline-deficient rats. Baby chicks, ducklings and turkey poults fail to grow on diets poor in choline and develop perosis (slipped tendon disease).[40,41] Trout fed a choline-deficient diet exhibited renal degeneration and intestinal hemorrhages.[42] Choline is necessary for the growth of the cockroach[43] and of mosquito larvae.[44] Many other effects of choline have been reported but there is not time for a complete listing of these.

Some of the lesions seen in choline deficiency are secondary to the renal damage, e.g., the ocular lesions[34,45] and the hypertension seen in rats.[46] Further work is advisable to determine whether the cardiac and vascular lesions reported by Wilgram and Hartroft[47] in 1955 are the result of a direct or indirect effect of choline deficiency. The edema observed by the Alabama workers[48,49] in rats fed diets low in both choline and protein deserves more study.

Certain biochemical changes are caused by lack of choline besides the well-known accumulation of glycerides and cholesteryl esters in the liver. The phospholipids in the liver are not decreased in absolute amount in cho-

line deficiency[50,51] but their turn-over is decreased.[52a,b,c] Adding choline to the diet, or injecting it, causes a prompt increase in the turnover of liver phospholipid-phosphorus but the effect is nonspecific, a similar increase being produced by methionine, ethanolamine or cystine.[53] Plasma phospholipids of rats are decreased in choline deficiency[51] and there is a marked reduction in the low-density lipoprotein fraction of the serum.[54,55] Liver damage due to prolonged lack of choline leads to a decrease in the concentration of cholesteryl esters in the blood serum of dogs,[7,56] and rats; [57,58,59] with advanced cirrhosis the concentration of total serum cholesterol falls very low. Restoring choline to the diet causes a prompt rise in the serum cholesterol, back to the normal level, long before there has been any significant improvement in the condition of the hepatic tissue.[59]

CLINICAL AND LABORATORY STUDIES

Much less is known about the role of choline in human nutrition.[60,61] Lipotropic studies in primates are not numerous. The original preliminary studies by Mann, Andrus, McNally and Stare[62] are being confirmed and extended in our laboratory. Dr. G. F. Wilgram has been following 4 cebus and 4 rhesus monkeys that have been fed a purified diet for several years. Two animals of each species have been on a choline-deficient ration, and two have been given choline-supplements. The monkeys getting choline grew well, indicating that the diet is adequate with respect to protein and calories. The choline-deficient monkeys grew only very slightly. When an exploratory laparotomy was performed after 6 and again after 12 months, the livers were seen to be fatty (by actual analysis of biopsy specimens, 14, 15, 19, and 20 per cent of total lipid) respectively. Histologic examination showed the same predominantly periportal lipid accumulation that is seen in human kwashiorkor. The fat within the liver cells appeared cytologically in a form very similar to that observed in human cases of protein malnutrition. Further experiments on monkeys, designed to extend this basic experiment and to test the curative effects of the lipotropic agents under conditions of paired feeding are in progress.

Several clinical conditions that are characterized by fatty livers and that are thought by most workers to be of dietary origin* are believed by some to represent clinical analogues of the lesions produced by dietary means in animals. It is generally stated that in patients with fatty livers resulting from alcoholism or kwashiorkor the distribution of stainable fat differs histologically from that produced in rats by choline deficiency: in the patients the distribution is largely periportal;[63] in the choline-deficient rat it is centrilobular.[64,65,66] We shall return to this subject of the clinical significance of choline after defining the term lipotropic and saying a few things about the lipotropic agents and certain lipotropic phenomena.

The term lipotropic was coined in 1935 by Best, Huntsman and Ridout[67] to describe the activity of dietary factors such as choline and betaine that prevent the accumulation of abnormal amounts of fat in the liver. Some years later Sellers, Lucas and Best[68] proposed that the coverage of the term be extended to include the hemorrhagic-kidney lesions produced in weanling rats by choline deficiency and the cirrhosis that develops in rats or dogs maintained for long periods on choline-poor rations. More recently, Wilgram, Hartroft and Best [69] proposed a further extension, to include the prevention of lipid deposition in other locations, specifically in the heart and blood vessels. The wisdom of this was questioned by some of our colleagues and one writer took vigorous exception to the proposal. Mann[70] would probably not have raised his objection if the various manifestations of choline deficiency had been discovered and reported simultaneously.[71]

Choline is the key substance, i.e., the ultimate effective agent in lipotropic phenomena. Betaine, methionine and to a lesser extent thetins serve as dietary precursors of choline. Choline is found ubiquitously in plant and

* A valuable collection of pertinent papers, experimental and clinical, dealing with nutritional factors and liver diseases, was published in *Ann. New York Acad. Sc.* 57: 615–962, 1954.

animal products. The richest dietary source of choline is egg-yolk which contains 15–17 mg/g; other rich sources are wheat germ, liver, brain, kidney, and heart.[60,72] Good dietary sources include lean meats, yeast, soya beans, and peanuts (much choline remains in the defatted meals), skim milk and milk powders. Fruits and vegetables are usually poor sources. It is difficult to design a diet low in choline from natural products, and to prepare a diet free from choline is costly. A ration devoid of choline precursors cannot be considered an adequate diet, a point we shall return to in a moment.

Information about the average daily intake of choline by man is meager. Estimates vary considerably but an intake of about 300 to 600 mg/day is probably common.[26,72] The choline requirement of man is not known. An absolute value probably cannot ever be determined since factors such as the methionine and cystine content of the diet, the amount of betaine and certain thetins in the food, the adequacy of several vitamins, particularly vitamin B_{12} and folic acid, as well as the type of bacteria in the intestine will all affect the choline requirement.

Large doses of choline chloride given to sheep and dogs in experimental studies[73,74] and to man in clinical studies have failed to increase the levels of either free or total choline in plasma. Only a very small fraction (under 10 per cent and usually 0.5 to 2.5 per cent) was recovered in the 24-hour urine. Approximately two-thirds of the dose of choline given orally to some normal adults was broken down to trimethylamine in the intestine[75] before it could be absorbed but no comparable urinary excretion of trimethylamine followed the intravenous administration of choline. Oral administration of antibiotics with oral choline temporarily reduced the excretion of trimethylamine, presumably by suppressing bacterial metabolism. Similar destruction of ingested choline by the intestinal bacteria of rats has been reported[76] but this has not been observed by all workers.[77] The different findings emphasize the possible importance of the gastrointestinal flora in the overall metabolism of choline in man.

Shortly after the lipotropic effect of choline and betaine[2] had been discovered in 1932, it became apparent that dietary proteins are also involved in lipotropic phenomena. The efforts of Professor Channon, at Liverpool, to account for this by testing various proteins and individual amino acids are well known (see reviews in references 22 and 72). In the year 1937 the sulfur-containing amino acid methionine was shown by Womack, Kemmerer and Rose[78] to be essential for the growth of rats and its lipotropic activity was discovered by Tucker and Eckstein.[79] Within three years the lipotropic effect of methionine was accounted for by Professor du Vigneaud and his colleagues[80] who found that its methyl group is biologically labile. For a time it appeared probable that the body cannot make methyl groups and that methionine provides this essential unit for the biosynthesis of choline. Later some limited synthesis of methyl groups was shown to occur in animals, and to be independent of bacteria in the gastrointestinal tract. The importance of vitamin B_{12} and of folic and folinic acids in the metabolism of the one-carbon unit and in the biosynthesis of methyl groups, and hence indirectly in lipotropic phenomena, has been established in the last decade.[81,82,83,84a,b]

METHIONINE

Methionine occurs almost as ubiquitously as choline.[85] Animal proteins as a rule contain more methionine than do plant proteins: lactalbumin 5.2 per cent, egg albumin 3.5, animal muscle powder 3.3, casein 2.8–3.0, fibrin 2.4, soya bean protein 1.0–1.2, defatted peanut meal 0.6, whole wheat flour 0.25–0.35, rice 0.25 and corn meal 0.2 per cent. Arachin, the globulin of peanuts, is noted for its low content (about 0.6 per cent) of methionine.[86] The use of defatted peanut meal (or soya bean meal or other pulses) permits one to design diets low in methionine and practically free from vitamin B_{12}. By extracting the meals with dilute ethanol (50 per cent v/v), or diluted methanol or propanol, followed by treatment with 80 per cent (v/v) and finally with warm 95 per cent alcohol, a product almost free from choline or betaine may be

obtained. (Dilution with water is essential to leach out the betaine and certain choline derivatives.)

Attempts to produce severely hypolipotropic diets, by extreme limitation of the methionine intake curtails not only the supply of labile methyl groups but reduces also the intake of amino acid sulfur and of the essential amino acid itself. Provision of supplementary cystine will correct the sulfur deficiency but only methionine itself can correct the essential amino acid deficiency.

If we accept the teaching that the nutritional value of a protein is limited by the essential amino acid present in lowest proportional amount, many of the basal diets that we and others have used in lipotropic work must be considered protein-deficient, in spite of the presence of from 15 to 25 per cent of protein in the food offered. In other words, it seems impossible to design a diet that is alipotropic, or even severely hypolipotropic, in the sense of being devoid of, or low in all choline precursors, without getting into protein deficiency. Apparently, an alipotropic diet that is otherwise adequate cannot be designed. Unsuspected protein deficiency doubtless has complicated many lipotropic studies.

The methionine requirement of man, in the absence of dietary cystine, has been estimated by nitrogen balance studies to be about 1.1 g/day and the recommended daily allowance has been set at double this figure.[87] Since normal diets contain considerable cystine, which spares methionine, the actual requirement for methionine is doubtless much lower.[88] Further, the presence of choline or of betaine or thetins in the diet will also lower the requirement. Thus the practical methionine requirement of even normal men is not known and that of patients with fatty livers, cirrhosis or other diseased states is still less clear.

BETAINE AND THETINS

Most articles on lipotropic phenomena give betaine and the thetins little attention, if they are mentioned at all. Yet betaine is of widespread occurrence in plants (sometimes in considerable amounts, up to 3 per cent of dry weight) and it has been found in a number of animal tissues, (malt, wheat germ, cottonseed press cake, leaves and roots of the ordinary red beet and of the sugar beet, various mushrooms and other plants, as well as in shrimps, crayfish, mussels, cuttlefish, and several mammalian tissues).[89] The methyl groups of certain thetins are labile[90,91] and hence these compounds, the occurrence of which in plant products appears to be more general than was previously suspected,[92,93] may play a minor role as dietary precursors of choline. The amounts of betaine and thetins in natural diets are usually unknown but they may, in some cases, contribute a considerable proportion of the total lipotropic supply.

In 1944 Beveridge, Lucas, and O'Grady[94] pointed out that the lipotropic activity of a protein is determined not only by its content of methionine and cystine, but also by the nature and amount of the other essential amino acids associated with them. This had been indicated by earlier studies of Channon and his colleagues but the evidence was less clear. More recently a number of papers,[23b] including several significant contributions from Dr. Sydenstricker's laboratory,[95,96] have elaborated this theme that dietary factors other than choline and its precursors affect the deposition of fat in the liver. Time will not permit a fuller presentation of the interesting findings in this field from the laboratories of Professor Sydenstricker, Professor Elvehjem,[97–101] Dr. Stewart here in New York and our own group, other than to mention that in rats fed diets with protein deficiency and amino acid imbalance the stainable fat appears first in periportal regions and rarely forms large droplets. The amount of total lipids extractable by hot alcohol is increased somewhat, but usually not greatly (possibly to about twice or three times normal). In choline deficiency the fat appears initially around the central vein; when the lack of choline is severe the lipids commonly form immense fatty cysts in the liver, and the amount of extractable fat is increased tremendously (three to eight times normal).

CLINICAL STUDIES

As mentioned earlier, the fatty livers with

or without cirrhosis seen in alcoholics and in children with kwashiorkor, seem to offer clinical analogues to the lesions produced by dietary means in rats, dogs, and other animals. It seemed reasonable, therefore, for the clinician to deduce that the lipotropic factors, whose efficacy in animal studies is well established, might provide specific therapy for corresponding pathological states in man. Optimistic reports followed the initial clinical trials of dietary supplements of choline and methionine. Recently, more cautious clinicians[61,102,103] have suggested that both the dietary and statistical control were inadequate in most of the early clinical trials. It is probably still true that no metabolic or clinical study has provided convincing proof that deficiency of choline or of methionine exists in patients with liver disease, although the circumstantial evidence is strong. In patients with alcoholism or kwashiorkor the distribution of stainable fat is largely periportal; in the choline-deficient rat it is centrilobular. Stainable fat does appear in the *periportal* areas of the livers of rats when they are fed diets deficient in protein. Thus if man responds to lipotropic deficiency as does the rat, the hepatic lesions attributable to nutritional disturbances commonly seen in the clinic, represent protein inadequacy, not choline deficiency. The frequently reported failure of choline to be of benefit in these cases would then be only what one would predict, since choline can be expected to cure only choline deficiency. However, to add a little information that may be confusing, we found, upon histologic examination of the livers of choline-deficient monkeys, the same predominantly periportal lipid accumulation that is seen in human kwashiorkor. Cytologically, the fat within the liver cells appeared in a form very similar to that observed in human cases of protein malnutrition. Thus the work on monkeys has not yet established with certainty the nature of the dietary defect causing the lesions seen in alcoholics and in patients with kwashiorkor. Dietary history and cytology implicate protein but do not exclude choline.

All clinical studies have failed to provide any evidence that supplementing an adequate hospital diet with choline, methionine, betaine or vitamins, brought about any more prompt or complete recovery of the fatty cirrhosis of alcoholics or of kwashiorkor than did the well-balanced diet alone. However, we in Toronto have long contended, and Gabuzda[61] has recently pointed out, that the failure of a lipotropic agent or other supplement to enhance the therapeutic value of a diet does not necessarily mean that the supplement is not concerned in the origin or treatment of the disease. It may mean that the hospital diet contains a maximally effective amount of the nutrient in question and hence one could not expect any further therapeutic effect.

The results of attempts to assess the therapeutic effect of lipotropic agents in man are difficult to evaluate. Clinical trials of the lipotropic agents have been made in patients with fatty liver disease due to kwashiorkor or to chronic alcoholism, usually under one of the following conditions:

(1) Supplementation of a diet already containing sufficient protein and lipotropic substances.

(2) Supplementation of a "purified" diet lacking any protein (intravenous or oral glucose with vitamins and minerals).

(3) Supplementation of a diet that had only a basic minimum of protein but that was too low in calories to permit therapeutic success.

None of these conditions can be considered satisfactory to establish the point in question. Only a very few cases have been studied in which lipotropic supplements have been given to patients maintained on an amino acid or a protein mixture that was kept just at a borderline level in amino acids and vitamins, and which was sufficient in calories. Only under these conditions could it be clearly shown whether or not lipotropic substances play a contributory part in the prevention of fatty livers in human subjects.

As experimentalists we would remind the clinician (1) that choline supplements to diets lacking methionine and other essential amino acids, are doomed to failure; (2) that when the methionine content of a diet is not ade-

quate, provision of choline spares some of the methionine for maintenance or growth; (3) when the supply of the lipotropic factors other than methionine is not adequate a portion of a supplement of this amino acid will be used for the synthesis of choline.

The factor or factors in complete diets that may account for their efficacy in curing fatty and cirrhotic livers in alcoholics, and liver damage in kwashiorkor, remain elusive. Certainly one would not exclude choline, betaine, or methionine. Clinical experience indicates that good natural protein is the most important factor and that it appears to carry along with it any requisite accessory factors.

In the light of all the experimental evidence it will be surprising if choline does not prove to possess lipotropic activity in man when the crucial test is made.

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The Development of Vitamin B_{12} Deficiency by Untreated Patients with Pernicious Anemia

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PRESENT evidence indicates that vitamin B_{12} is identical with the red cell maturation of Castle and with at least one of the so-called extrinsic factors. Gardner and his co-workers[2] postulate that pernicious anemia is due to a deficiency of vitamin B_{12} conditioned by the lack of gastric intrinisic factor which is essential for the absorption of the vitamin. This hypothesis has much support. Particularly convincing is the evidence presented by Welch and his co-workers[4] demonstrating that normal gastric juice enhances the disappearance from the gut of labeled cobalt ingested as vitamin B_{12} by subjects with pernicious anemia. The relapse which follows the withholding of maintenance therapy from subjects with pernicious anemia may be considered, therefore, as the development of a vitamin B_{12} deficiency state. Hence, a study of the characteristics of the developing deficiency state should be of interest to both hematologists and nutrition workers.

Before the advent of liver therapy for pernicious anemia the occurrence of spontaneous remissions, at times of some years' duration, was observed.[5] Furthermore, relapse after cessation of treatment is gradual and may vary in its course.[6,7,8] Knowledge of the characteristics of this relapse is necessary for interpreting studies of the hematopoietic activity of such agents as crystalline vitamin B_{12}, folic acid (pteroylglutamic acid), or citrovorum factor, particularly when appraising the ability of these substances to maintain hematologic levels of patients who are already in remission.

This report describes the course of relapse following interdiction of therapy in previously well-maintanied patients with pernicious anemia. It indicates that the time required for depletion of previously well-maintained patients is longer than widely held opinion would indicate.

CLINICAL MATERIAL AND METHODS

The present report gives additional observations on the group of patients studied by Jones, Tillman and Darby.[8] Between November, 1945 and January, 1946, we discontinued the administration of liver extract to 13 patients on whom the diagnosis of pernicious anemia had been made. When first seen, most of these patients had given a history of anemia or weakness of several years' duration. A story of dramatic response to oral or parenterally administered liver extract was frequently elicited. Eleven patients had been demonstrated to have gastric achlorhydria after histamine injection, and liver therapy had evoked a reticulocyte crisis and a rapid increase in erythrocyte count. In eight of these the bone marrow had been examined during a relapse and described as megaloblastic. These 11 patients therefore fulfilled rigid diagnostic criteria of pernicious anemia. One patient originally included in the study has been elim-

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inated for lack of sufficient evidence for the diagnosis. Another patient (P. H.) when initially seen gave a history of anemia, paresthesias, and ataxia, which had responded to liver therapy administered by her local physician. She was in remission when first seen and was maintained by liver therapy in our clinic. Her inclusion in the series is justified by the demonstration of histamine-fast achlorhydria and the occurrence of a relapse during the period of the study. Table I summarizes pertinent data relative to signs, symptoms, and previous therapy in the group of 12 patients followed in the study.

As discussed in the earlier paper,[8] in several instances adequate and often intensive liver extract therapy had failed to raise the red cell counts to the level of 4.5 million/cu mm considered by many as normal. Hence, a normal or standard hematologic value was determined for each individual as the average of the values measured during the period of maximal maintenance prior to omission of therapy. These maximum average levels during treatment with liver extract are shown in Table I. A relapse was defined as the occurrence of at least two successive erythrocyte counts at intervals of a week or more, which were two standard deviations or greater below the average level for the patient during periods of adequate therapy.

Most of the patients were seen at least once monthly during the period without therapy. Blood counts, measurements of body weight, and examinations of the tongue and nervous system were made, so that the course as well as the time of relapse could be observed.

A second group of ten individuals who had been observed in a relapse after self-imposed interdiction of therapy is included. This group was obtained by surveying the records of 75 patients with well-established pernicious anemia seen in this clinic within the last 15 years. The development of these relapses was not observed, due to absence of the patient from the clinic. When the patients returned because of symptoms their relapses were advanced. Hence, the length of time required for these relapses is somewhat overestimated in comparison with the time recorded for the early stage of relapse seen in those patients who had been under continuous observation.

CASE REPORTS*

CASE 1. J. T., 36-year-old white male, had anemia with glossitis and diminished vibratory sense at the ankles in 1936. Parenteral liver extract was given for 51 months. Early relapse occurred three months after self-imposed cessation of therapy and treatment was then resumed for 53 months. During the latter part of this period therapy was somewhat irregular and he began to show macrocytosis and a diminishing erythrocyte count before treatment was stopped, thus indicating inadequate treatment. Relapse occurred four months after cessation of treatment. Glossitis recurred in each relapse and was relieved by therapy. Neurological signs disappeared during the initial period of therapy and did not reappear.

CASE 2. H. C., 44-year-old white male, entered the clinic in 1945 with a one-year history of anemia. Liver extract was given for nine months, following which the interdiction of therapy resulted in a relapse within five months. Two months later a spontaneous remission of four months' duration began. A second relapse with macrocytosis then occurred, the erythrocyte count falling to 2.5 million within 15 months from the time treatment was omitted.

CASE 3. F. L., 42-year-old Negro male, first noted weakness and paresthesias in 1936. These responded to dietary management (liver) but he failed to continue the diet and was admitted to the hospital in extremis one year after anemia began. He was treated for about four months, then received liver extract infrequently from his local physician, returning in relapse some three years after regular injections of liver extract had been stopped. Treatment was resumed for five years and then withheld. He relapsed within nine months, but his red cell count did not reach 2.5 million for 28 months after cessation of therapy.

CASE 4. C. T., 70-year-old white male, had been treated for 50 months for anemia complicated by bilateral thrombophlebitis of the legs. Therapy was suspended in 1945. An initial relapse began in nine months and was followed three months later by a spontaneous remission. Relapse recurred at 17 months after cessation of therapy and his erythrocyte count decreased to 2.5 million at 35 months. His erythrocytes were slightly macrocytic even while he was being treated, but macrocytosis increased after therapy was stopped.

CASE 5. M. C., 64-year-old white female, had noted

* Ages given are those of the patients when therapy was discontinued December, 1945–January, 1946; periods of time, unless otherwise specified, begin with the cessation of therapy. Macrocytosis is defined as a persistent MCV > 100 cu μ.

TABLE I

Summary of Case Presentations*

Patient	Presenting symptoms	Therapy				Time before onset of										Remarks
		Duration			Average maximum maintenance RBC	Macrocytosis†		Relapse		RBC of 2.5 million		Auxiliary symptoms			Spontaneous remission	
Color, Sex, Age		Yr.	Mo.	Type‡	Mean ± S. D.	Yr.	Mo.	Yr.	Mo.	Yr.	Mo.	Type	Yr.	Mo.	Yr.	Mo.
J. T., WM, 63	Glossitis	4	3	L. E.				—	3	—	—	Glossitis	—	3	—	—	
	Paresthesia					During											
	Weakness	4	5	L. E.	4.36 ± 0.22	therapy		—	4	—	—	Glossitis	—	4	—	—	Irregular therapy
H. C., WM, 44	Weakness	—	9	L. E.	4.11 ± 0.24	—	—		5	—	—	None	—	—	—	7	
		—	—	—		—	11		11	1	3	None	—	—	—	—	—
F. L., CM, 42		—	—	O. L.	4.68 ± 0.24			1	—	—	—	—	—	—	—	—	Some L. E. in interval
	Weakness	—	4	L. E.				3	—	—	—	None	—	—	—	—	
	Paresthesia	5	—	L. E.				—	9	2	4	None	—	—	—	—	
C. T., WM, 70	—	4	2	L. E.	4.47 ± 0.17	During		—	9	—	—	None	—	—	1	—	Thrombophlebitis during therapy
						therapy		1	5	2	11	None	—	—	—	—	
M. C., WF, 64	Glossitis	—	6	O. L.	4.66 ± 0.25	—	—	—	6	—	—	—	—	—	—	—	—
	Weakness	7	10	L. E.		—	10	—	10	2	10	Glossitis	2	3	—	—	
J. J., WM, 48	Weakness	9	8	L. E.	4.41 ± 0.32			1	1	2	7	Glossitis	1	7	—	—	Healed gastric ulcer
												Paresthesia	2	7	—	—	
C. T., CM, 44	Weakness	5	5	L. E.	4.94 ± 0.36	—	—	3	1	—	—	Neurologic	3	1	—	—	Absent from clinic
	Paresthesia	1	9	L. E.		1	6	1	6	—	—	None	—	—	—	—	— —
		—	8	B_{12}		—	—	—	6	—	—	None	—	—	—	—	2.5 μg/2 weeks
		—	5	B_{12}		—	—	—	7	—	—	None	—	—	—	—	5.0 μg/2 weeks
		—	0	B_{12}		—	—	—	8	—	—	None	—	—	—	—	Single 45 μg
J. H., WM, 65		—	1	L. E.	4.38 ± 0.24	—	—	—	10	—	—	—	—	—	—	—	
	Weakness	—	1	L. E.		—	—	—	9	—	—	Neurologic	—	9			
		—	?	L. E.		—	—	—	8	—	—	Neurologic	—	8			
		5	8	L. E.		1	—	2	5	4	6	None	—	—			
A. P., WF, 60	Weakness	4	4	L. E.	4.21 ± 0.06	2	1	3	2	—	—	—	—	—	4	7	Macrocytosis persisted
						—	—	4	9	5	10	Glossitis	5	4	—	—	
E. T., WM, 72		—	2	O. L.	4.88 ± 0.27	—	—	1	—	—	—	Paresthesia	1	—	—	—	Pleural effusion RBC never below 3.9
	Paresthesia	5	9	L. E.		2	10	3	4	—	—	None	—	—	3	9	
						—	—	6	2	—	—	None	—	—	—	—	
C. H., WF, 52	Paresthesia	5	11	L. E.	4.10 ± 0.32	3	4	5	3	—	—	Glossitis	5	6	—	—	Urinary infection, gallstones
P. H., WF, 60	Paresthesia	10	9	L. E.	4.42 ± 0.36	5	5	6	3	—	—	None	—	—	—	—	First seen in remission

* All periods of time begin with the cessation of therapy, except for "Duration of Therapy." Ages are those at the time of cessation of therapy in 1945.

† Macrocytosis is defined as a consistent finding of MCV > 100 cu μ.

‡ L. E. = Liver extract. O. L. = Oral liver.

glossitis, weakness, and jaundice in 1936 and her symptoms responded to six months of oral liver extract therapy. She came to the clinic for the first time in 1937, in relapse, and was treated for 94 months. Relapse and macrocytosis occurred 10 months after suspension of treatment but her red count did not fall to 2.5 million for 34 months. Glossitis recurred 27 months after cessation of therapy.

CASE 6. J. J., 48-year-old white male, had noted weakness and prostration in 1935 and therapy with liver extract was initiated in 1936 and continued for 116 months. Vague paresthesias occurred during therapy. Following interdiction of therapy hematologic relapse began in 13 months and his red count reached 2.5 million in 31 months. The tip of his tongue became red and sensitive in 19 months and paresthesias recurred in 31 months.

CASE 7. C. T., 44-year-old Negro male, had noted the onset of weakness and paresthesias in 1932. These symptoms responded more or less well to a poorly followed liver diet. Treatment with liver extract was begun in 1935 and continued for 65 months. He then left the clinic to return in 1944, 37 months later, in relapse with neurologic symptoms. Treatment was resumed for 21 months and neurologic symptoms have never recurred. Following cessation of therapy hematologic relapse and macrocytosis began in 18 months. He was treated in 1949 with 2.5 μg of crystalline vitamin B_{12} given intramuscularly every two weeks for eight months. He left the clinic and returned in relapse in six months. Treatment was resumed with 5 μg of vitamin B_{12} every two weeks for five months. He again left the clinic but returned in relapse in seven months. He was treated by a single injection of 45 μg of B_{12}, left the clinic, and returned in relapse in eight months (See Figure 2).

CASE 8. J. H., 65-year-old white male, had noted weakness in 1936 and was found to have anemia in 1937. He was treated with a total of 35 cc of concentrated liver extract in several injections. He left the clinic to return 10 months later in relapse. The dosage of 35 cc of liver extract was given again. He failed to return until nine months later when he appeared in relapse with early combined system disease. Small doses of liver extract were then given and he was transferred to a local physician who gave him an unstated amount of liver extract. He returned in relapse with advancing combined system disease eight or nine months after his last injection. Treatment was resumed in 1940 for 68 months. His neurologic symptoms improved during therapy and have shown no further exacerbations. Following withdrawal of maintenance therapy macrocytosis occurred in one year and relapse began at 29 months. His red count reached 2.5 million per cu mm in 54 months.

CASE 9. A. P., 60-year-old white female, had developed weakness which responded to injections administered by her local physician in 1940. Her symptoms recurred in 1941 and were treated with liver extract for 52 months. Following the withdrawal of maintenance treatment macrocytosis began in 25 months and relapse at 38 months. A spontaneous remission which lasted two months began 55 months after the injections of liver extract were stopped, but macrocytosis persisted throughout this period. Her tongue became red and sore 64 months after cessation of therapy, but the red count did not fall to 2.5 million per cu mm until 70 months.

CASE 10. E. T., 72-year-old white male, had been found in 1939 to have anemia with combined system disease and was treated with oral liver by his local physician for about two months. Anemia and paresthesias recurred in 1940, one year after cessation of diet therapy. Both responded to treatment with liver extract which was continued for 69 months, at which time therapy was interdicted. Macrocytosis appeared at 34 months and relapse occurred at 40 months. Spontaneous remission occurred at 45 months and a second relapse at 74 months. This latest relapse persisted three months, and macrocytosis persisted from its first occurrence until 56 months after cessation of therapy. The second spontaneous remission has continued to date, and at no time has the erythrocyte count dropped below 3.9 million per cu mm.

CASE 11. C. H., 52-year-old white female, with a history of chronic mild urinary tract infection, had been found to have anemia with early combined system disease in 1940. The presence of gallstones was demonstrated by x-ray. She was given liver extract for 71 months. Following cessation of therapy, her red count decreased somewhat in about two years, but did not fall to the relapse level until after 63 months. Macrocytosis began at 40 months, glossitis appeared after 66 months, but neurologic symptoms did not reappear.

CASE 12. P. H., 60-year-old white female, had developed anemia with neurologic symptoms in 1923 and was treated with liver extract by her local physician for three years beginning in 1935. In 1938 she came to the clinic in remission, and liver extract was continued for 93 months. Maintenance treatment was withheld and macrocytosis appeared at 65 months and hematologic relapse began at 75 months. Neurologic symptoms have not recurred.

RESULTS

All of the 12 patients studied suffered relapses within 78 months and 11 have been treated and have responded to specific therapy with vitamin B_{12} in a diagnostic manner. Figure 1 shows the time required for relapse in each of these patients and the occurrence of spontaneous remissions. No therapy was given to any patient during the time depicted in the

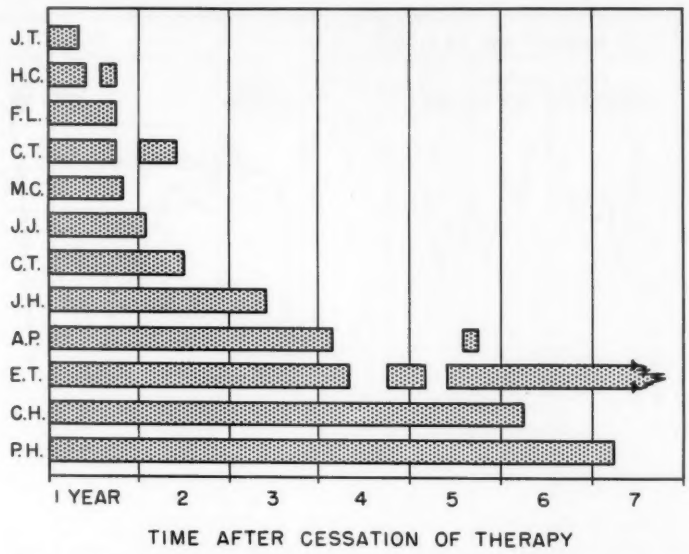

Fig. 1. Time required for relapse of patients with pernicious anemia after cessation of treatment with liver extract. Stippled areas represent periods of time during which the patient's erythrocyte count was normal. H. C., C. T., A. P., and E T. experienced spontaneous remissions.

graph. Liver was excluded from their diet. All of these patients disliked liver (some because of having been required earlier to eat large quantities) so that this dietary restriction was of no inconvenience to the patient.

Spontaneous remissions occurred in 4 of the 12 patients—two such remissions in one subject. In no instance was a spontaneous remission preceded by profound relapse and only one remission proved to be of more than a few months' duration. The ultimate rate of spontaneous remission cannot be estimated as our patients were not allowed to relapse to a level appreciably below an erythrocyte count of two million prior to institution of specific therapy.

In each of the 16 observed relapses in these 12 patients the hematologic values declined gradually over a period of months. Even when a spontaneous remission did not occur the progressive decrease in erythrocyte values to 2 to 2.5 million per cu mm required some months after the beginning of the relapse. The onset of macrocytosis was frequently noted several months prior to the fall in red cell counts to the relapse level and persisted at least until sufficient therapy was administered to return the erythrocyte count to well within the zone of the average normal red cell count. This finding indicates that macrocytosis may serve as a sensitive index of therapeutic adequacy, a point which is considered further in a continuation of our group during a long period of therapy with minimal dosage of vitamin B_{12}. The pattern of hematologic relapse is well illustrated by the course of patient C. T. (Fig. 2) during the interval from November, 1945 to early 1949. This pattern is similar to that observed for other patients in the series, some of whom were previously depicted.[8]

Combined system disease did not develop in any of the 12 patients during the period without therapy. The only finding suggestive of neurologic involvement during the period of controlled relapse was in one patient (J. H.) who had symptoms of mild tingling in the legs after hematologic relapse was well advanced. This is in contrast with the record which revealed that at their initial admission to the hospital in relapse six of these patients had definite early combined system disease and two

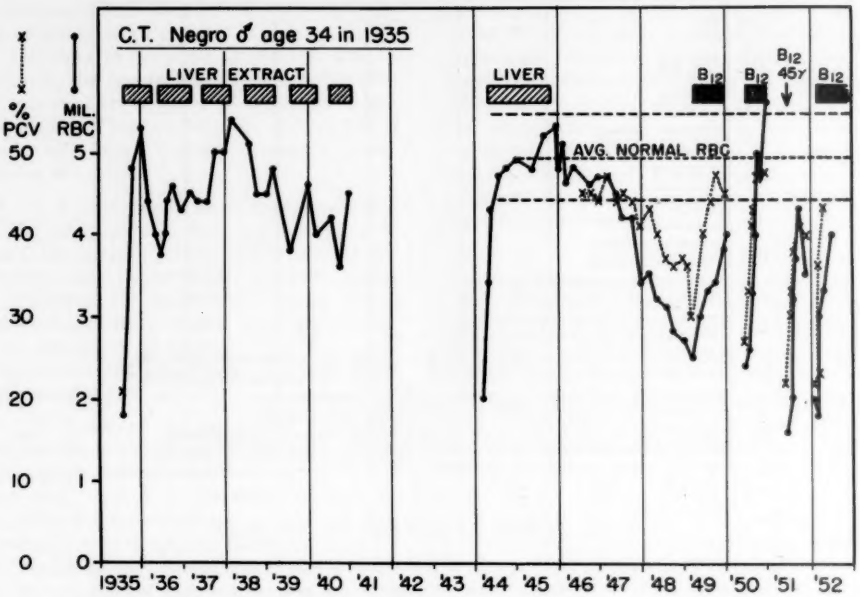

Fig. 2. Hematologic values of a patient with pernicious anemia during periods with and without therapy. The scales are such that when the erythrocyte count and the packed cell volume coincide, the mean corpuscular volume is 100. Therefore, when the PCV is above the RBC, the cells are macrocytic. The three horizontal broken lines represent his normal red cell count determined in 1945 and the range included within two standard deviations above and below normal.

others had suggestive paresthesias. In successive unplanned relapses some of the patients experienced advancing neurologic changes. All of these symptoms had improved or disappeared during the period of intensive therapy prior to interdiction of treatment. These observations indicate that hematologic relapse as defined in this paper may be expected to precede the appearance of neurologic changes as subjects become deficient in vitamin B_{12}. It is recognized that instances are encountered of untreated combined system disease which respond to the liver factor but are characterized by the absence of appreciable anemia. From our studies, it seems that such patients may actually be in hematologic relapse as here defined, but that the severity of the hematologic changes are unimpressive. More careful evaluation of the size of the erythrocyte through measurement of the mean cell volume in these patients may be useful in detecting early hematologic relapse. These observations are not consistent with a widely-held opinion that the occurrence of combined system disease is indicative of an especially large need for the hematologic factor. They suggest, however, that the patient with hematologic signs is severely depleted.

Changes in body weight were unexpectedly inconsistent. Most patients exhibited a strikingly constant weight throughout the long period of observation irrespective of status as to relapse or remission; two subjects gradually but constantly gained in weight throughout the period of study despite the appearance of moderately severe hematologic relapse. In only four cases did there occur a decrease in weight simultaneously with the hematologic relapse. In only one instance was the loss greater than 10 pounds and in this case it amounted to 21 pounds. Where loss of weight was observed it coincided with the development of the hematologic relapse and was equally gradual of development.

The occurrence of auxiliary symptoms in our series was singularly lacking. In five of the

TABLE II

Time Elapsing Between Self-Imposed Discontinuance of Therapy and Return for Resumption of Treatment by Patients with Pernicious Anemia

Patient	Sex	Age at discontinuance of therapy	Therapy	Duration of therapy		Time for relapse		Remarks
				Yr.	Mo.	Yr.	Mo.	
T. G.	M	65	255 u L. E.*	0	1	0	5	Later developed carcinoma of the stomach.
A. H.	F	71	570 u L. E.	1	7	0	6	Last 18 months of therapy probably inadequate.
H. P.	M	47	Liver diet	2	1	0	9	Did not adhere to diet.
A. W.	F	54	340 u L. E.	0	0.5	0	11	Combined system disease appeared during earlier treatment with folic acid.
F. K.	M	57	Weekly L. E.†	1	0	1	2	
J. R.	M	70	137 u L. E.	0	1	1	6	
H. L.	M	67	Weekly L. E.†	2	0	2	6	
L. G.	F	68	130 u L. E.	0	2	3	1	
		71	15 mg/day folic acid	0	4	1	8	Good initial hemopoietic response to folic acid.
R. L.	M	59	Oral L. E.	5	0	4	0	Remained in relapse 4 years without treatment.
M. R.	F	45	450 u L. E.	1	4	5	10	Subsequent response to folic acid complicated by combined system disease.

* u L. E. = units liver extract.

† Unitage of liver extract unknown.

patients malaise, weakness and a sore, red tongue occurred, but not until hematologic relapse had been present for several months. At the time of onset of defined relapse the patients were uniformly asymptomatic.

In addition to the controlled relapses, 10 unplanned relapses had occurred in four of these patients prior to 1946. These are detailed in the case reports. These relapses were not observed until symptoms forced the patients to seek further treatment, and therefore the time required for their occurrence is overestimated. They serve, however, as an independent series against which our controlled observations may be viewed.

Twelve additional relapses were observed in 10 other patients whose records were reviewed, although the exact details of the occurrence of the relapse are unknown. These data are summarized in Table II and demonstrate great variability in the time required for symptoms to cause the patients to seek medical aid after cessation of therapy. This time ranged from 5 to 70 months, periods similar to those observed in our group which was under continuous observation.

DISCUSSION

One of the most remarkable findings in this study is that hematologic relapse uniformly occurred several months before the patient noted any symptoms of pernicious anemia (combined system disease, glossitis, diarrhea, or weakness). It appears that this type of study carries little risk of allowing irreparable neurologic damage to occur. This may indicate that efforts at the production of vitamin B_{12} deficiency in normal human subjects under conditions of careful frequent observations may be undertaken with relative safety.

Relapse may be exceedingly slow in developing after cessation of therapy in pernicious anemia. This has been recognized before, but reference to Table III demonstrates that our group required considerably longer to relapse than have previous series of patients.[5,6]

TABLE III

Comparison of Time Required for the Relapse of Patients with Pernicious Anemia Following the Withdrawal of Therapy

Months required for relapse	Cabot's series[5] (329 cases) %	Strauss and Pohle's series[6] (12 cases) %	Present series (12 cases) %
1–3	24	18	0
3–6	25	26	15
6+	51	56	85

This probably reflects the more rigid criterion of relapse which we have employed.

Slow relapses and the lack of early auxiliary symptoms in our patients raise the question of whether our group is representative of patients with the disease. The sex and age distribution of our patients, plus the fact that more than half of them had glossitis and combined system disease at some time during the course of their anemia, indicate that they were not an atypical group. The adequacy of our criterion for relapse is suggested by the fact that in only one patient (E. T.) was a beginning "relapse" not followed by an eventual fall of the red count to quite low levels. Macrocytosis occurred either simultaneously with or even some months before the onset of relapse. This indicates that macrocytosis is a useful early sign of relapse and a sensitive indicator of inadequate therapy. Our limited data indicate that macrocytosis occurred before the rise in fecal urobilinogen.[8] This relationship deserves further study for it may shed light on the question of whether increased blood destruction is due to the production of large abnormal cells which are more easily destroyed.

It is of interest to try to explain the variability in time required for clinical and hematologic relapse. This clinic and others[6,7,8] have attempted to correlate the variability with the amount of liver extract given during prolonged treatment. No correlation has been found and these studies appear to show merely that some patients have higher requirements for liver extract than others. Unpublished data from this clinic on the treatment of pernicious anemia with minimal doses of vitamin B_{12} confirm this variability in requirement.

It seemed that analysis of successive relapses in individual patients might be more profitable. Figure 2 shows the course of several relapses in one patient and demonstrates that in this subject longer remissions followed the periods of more intensive treatment. The quantity of therapy administered during the first several years was apparently inadequate to maintain his erythrocyte count at the higher level established as "normal" during the second period of treatment. It may be seen that macrocytosis preceded relapse and was abolished by therapy after the red count reached normal levels. Table IV presents three patients in whom successive remissions seemed to vary in duration with the quantity of previous therapy.

These observations may be interpreted as indicating that considerable storage of vitamin B_{12} occurs and that the amount stored is one of the factors which determines the duration of a remission following the cessation of specific therapy. This interpretation does not, however, explain the occurrence of spontaneous remissions. We may speculate that these remissions could reflect a "release" of previously stored but unavailable vitamin B_{12}.

The great variation in time required for relapse can reflect inherent differences in requirement and rate of utilization of the vitamin, differences in quantities of vitamin B_{12} stored, or the absorption of varying small quantities of the vitamin due to the secretion of varying appreciable, but inadequate quantities of intrinsic factor.[9,10]

When it became possible to use Co^{60}-labeled vitamin B_{12} in the study of absorption, there were still available to us three of the patients who relapsed in less than one year, and four whose relapses did not occur until three or more years without treatment. Using the technic of Heinle, Welch, *et al.*[11] for measurement of absorption, these groups showed an average fecal loss of Co^{60}-labeled vitamin B_{12} of 77 per cent, respectively; based on a single test for each patient. Hence, the observed differences in relapse time could not be attributed to differences in absorption of vitamin B_{12} due to effective quantities of intrinsic factor present.

The differences in relapse time most probably reflect the difference in body stores and

TABLE IV

Patients with Pernicious Anemia Exhibiting a Relationship of Therapy to Time Required for Relapse

Patient	Therapy				Time required for relapse	
	Units of L. E.* or μg of vitamin B_{12} injection	Intervals (weeks) between injections	Duration			
			Years	Months	Years	Months
C. T.	30–50 L. E.	1–2	5	6	3	1
	30 L. E.	2	1	9	1	6
	2.5 B_{12}	2	0	8	0	6
	5 B_{12}	2	0	5	0	7
	45 B_{12}	Single injection	—	—	0	8
J. H.	L. E., 350 units total	—	0	1	0	10
	L. E., 350 units total	—	0	1	0	10
	L. E., 200 units total	—	0	2	0	9
	50 L. E.	4	5	8	2	5
E. T.	Liver diet	—	0	2	1	0
	20–30 L. E.	1	5	9	3	4

* L. E. = liver extract.

rate of utilization of the vitamin. The data of Table IV are consistent with the interpretation that storage of the vitamin may vary greatly, and thereby alter the time required for depletion.

Experimental dietary vitamin B_{12} deficiency has not been produced willfully in the adult human. Observations on vegans,[12] however, indicate that a syndrome of glossitis, neurologic changes, and anemia does occur in some persons after long adherence to the vegan regime. This is associated with low serum levels of vitamin B_{12} and appears to respond to the administration of this vitamin. The vitamin B_{12} content of the diet on which the syndrome occurs has not been well established, but it appears to be very low. The absence of the syndrome in vegans in the U. S., in contrast to Europe, has been postulated as due to differences in intestinal synthesis of the vitamin in the two situations. Wokes, *et al.*[13] have noted a relationship between the serum vitamin B_{12} levels of their vegans and the length of time the subjects had followed the dietary regime.

Development of macrocytic anemia in patients following total gastrectomy has long been an accepted clinical occurrence, and has been attributed to removal of the source of intrinsic factor and thereby the precipitation of a deficiency of extrinsic factor. Studies of the altered absorption of vitamin B_{12} by gastrectomized patients confirm this hypothesis.[14–17] Paulson and Harvey[18] studied 27 patients who, following gastrectomy, slowly developed hematologic changes of macrocytosis (six months to seven years), anemia (one to eight years), and megaloblastic marrow (two to seven years), in that order. The macrocytic megaloblastic anemia may occur within a few months, but usually manifests itself some two years or more after operation. The variance in the development of the syndrome has puzzled some observers.

It is instructive to compare the relapse of patients with pernicious anemia to the development of the syndrome in vegans and in gastrectomized subjects. In our group the time required for relapse under observation varied from 4 to 75 months, a range almost exactly comparable to that required by gastrectomized patients to develop vitamin B_{12} deficiency and comparable to, although slightly less than, that required for vegans to develop their signs of deficiency. In our second group of relapses occurring without continuous observation, the "relapse time" varied from 5 to 70 months. Although the time of relapse so judged was somewhat overestimated, the variable periods required were still comparable to that of the groups who had been rendered deficient by other procedures (dietary or gastrectomy).

It seems unlikely that these three different groups with induced vitamin B_{12} deficiency

should all exhibit a similar aberration of requirement. Rather this would seem to reflect normal variations in storage and utilization of the nutrient. Such reasoning favors the conclusion that the patient with pernicious anemia can be considered a suitable subject for observations on the vitamin B_{12} requirement of man. Observations based on such patients, in whom minimal and subminimal initial maintenance dosages of the vitamin have been titrated, lead us to suggest that the minimal daily combined utilization, destruction and loss of absorbed vitamin B_{12} is in the region of 0.5 μg. If the healthy adult absorbs on the average some 70 per cent of the ingested vitamin at normal dietary levels, the daily requirement may be in the neighborhood of 0.7 μg. The evidence for, and further discussion of these tentative levels will be published separately.

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END OF SYMPOSIUM

Clinical Reports

Nutritional Studies of Vegetarians

III. Dietary Levels of Fiber

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AN INVESTIGATION of the nutritional, physical, and laboratory findings, together with the dietary and serum levels of cholesterol, of 88 non-vegetarian, 86 lacto-ovo-vegetarian, and 26 "pure" vegetarian adults, adolescents, and pregnant women was reported earlier.[1,2] Lacto-ovo-vegetarians include milk and eggs in their diet but do not eat flesh of animals (meat, poultry, fish). "Pure" vegetarians eat no food of animal origin. The details concerning the selection and composition of these groups are described in the preceding papers. The present study deals with the fiber content of the diets of these several groups.

The food composition tables of the U. S. Department of Agriculture[3] provided most of the data for the computation of the fiber content of the foods consumed. In a few instances information was also obtained from other sources.[4,5]

FINDINGS

As summarized in Table I, the fiber intake of the lacto-ovo-vegetarians was nearly twice that of the non-vegetarians and a little less than half that of the "pure" vegetarians.

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An examination of the diets reveals that the high fiber intake of the pure vegetarian diets was due to the unrefined, natural character of the foods. The calories and protein were derived mainly from whole grain cereals, legumes, and nuts, and some from oily, nut-like seeds as sunflower and sesame. Characteristic of this group was the consumption of unusually large amounts of nuts and nut butters, and of fresh, dried, and canned fruits. Vegetables and legumes were also eaten in considerably greater quantities than by either of the other two dietary groups. Some individuals included very large raw vegetable salads in their diets. Almost every food used contributed some fiber. The consumption of foods low in fiber as white sugar, white flour, refined cereals, customary desserts, and commercially prepared foods was at a minimum, if not wholly lacking in the pure vegetarian diets.

In general the food pattern of the lacto-ovo-vegetarian parallels that of the average American except for the absence of flesh foods. Dairy products and eggs, both lacking in fiber, provided much of the protein and approximately 20 per cent of the calories. This group was not as strict in the non-use of white sugar, refined foods and commercial preparations as were the pure vegetarians. However, they did consume considerable quantities of legumes, nuts, whole grain cereals and dark bread as well as fruits and vegetables of all kinds. Because these diets included both fiber-free and low-fiber foods, the total fiber intake was significantly less than that of the pure vegetarians.

The diets of the non-vegetarians were the

TABLE I

Comparison of Fiber Intakes of Vegetarian and Non-Vegetarian Groups

Groups	No.	Calories	Fiber			
			g/day		mg/100 cal	mg/kg
			Mean	S.D.*	Mean	Mean
Adults						
Males						
L-o-vegetarian†	15	3020	16.3	9.3	537	220
Pure vegetarian	14	3260	23.9	7.0	787	362
Non-vegetarian	15	3720	10.7	3.3	288	139
Females						
L-o- vegetarian	15	2450	12.6	9.3	515	201
Pure vegetarian	11	2400	20.7	7.3	857	390
Non-vegetarian	15	2690	8.4	1.9	313	131
Adolescent						
Males						
L-o-vegetarian	15	4450	17.8	8.2	399	278
Non-vegetarian	15	5350	12.2	3.2	228	192
Females						
L-o-vegetarian	15	3030	12.9	7.3	417	242
Non-vegetarian	15	4100	10.6	2.2	257	208
Pregnant women						
L-o-vegetarian	26	2650	12.4	11.1	467	210
Non-vegetarian	28	3010	8.4	5.4	282	144

* Standard Deviation.
† Lacto-ovo-vegetarian.

lowest in fiber content, yet well above Cowgill's[6] estimate of daily fiber requirement of 90 to 100 mg/kg of body weight, or approximately 6 g for an adult. Meat, milk, and eggs, foods almost devoid of fiber, were the main sources of protein and contributed approximately 30 per cent of the total calories. The free use of sugar and other refined and processed foods, added but little fiber to the diet. However, the amounts of fruits and vegetables consumed, though less than that of either of the preceding groups, more then met the usually recommended dietary pattern.

DISCUSSION

From time to time the question is raised as to the compatibility of a diet high in crude fiber with the normal functioning of the human digestive system. It is of interest that the regimes of the two vegetarian groups, with their large intake of fiber, had been maintained for long periods of time. The pure vegetarian males averaged 16 years and the females 9 years on their diets, with a minimum of 5 years for any subject studied. The lacto-ovo-vegetarian groups had, except for a few of the pregnant women, consumed their dietaries throughout life. Some had a history of this pattern of nutrition for two and three generations.

Subjectively, neither vegetarian group revealed any complaints relative to the digestive system. Even the large fiber intake of the pure vegetarians caused no alimentary disturbance. Since even the lower level of fiber obtained by the non-vegetarian groups was in excess of Cowgill's estimated requirement, it is not surprising that constipation was practically unknown among any of the groups. While the fiber content *per se* does not necessarily indicate the total bulk of the stools,[7–9] it may contribute materially to it. Unfortunately, the circumstances under which this study was done made it impractical to measure stool volumes.

The previous report[2] of the study of these groups showed that the levels of serum cholesterol of the adults were appreciably lower in the pure vegetarians (average 206 mg/100 ml) than in the non-vegetarians (average 291 mg/-

100 ml), with the lacto-ovo-vegetarians having intermediate values (average 256 mg/100 ml). Body weights varied somewhat in proportion to the level of cholesterol, but dietary fat intake did not, the lacto-ovo-vegetarians and pure vegetarians having essentially the same total fat intake as the non-vegetarians, though more of the fat was from dairy and/or vegetable sources.

It is possible that the increased fiber content of the vegetarian diets reported here may have played some role in the lower cholesterol levels observed, though we have no direct evidence of this. Certainly one would expect the fiber content to affect markedly the bacterial flora, which in turn might affect cholesterol metabolism. People on low-fat diets in whom lower cholesterol levels have frequently been reported usually also have diets high in fiber.

SUMMARY AND CONCLUSIONS

The results of this study indicate that lacto-ovo-vegetarians consume significantly more fiber than non-vegetarians, and pure vegetarians significantly more than lacto-ovo-vegetarians.

The generous intake of fiber in the diet of vegetarians for long periods of time and even for lifetimes, appears wholly compatible with normal functioning of the gastrointestinal tract. Since constipation was practically unknown in all dietary groups, it would appear that even the lower fiber content of the non-vegetarian groups was sufficient for physiologic needs. The possible relationship between fiber content of these diets and cholesterol levels is pointed out.

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Metabolic Studies of Mongoloids

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DURING the hundred years that mongolism has been recognized as a medical entity, many studies of the disease have been made. The epidemiology of this condition has received considerable attention and much emphasis has been placed on the anthropometric, physical, and mental characteristics of the mongoloid. There is, however, a marked lack of data concerning the metabolism of mongoloids and those which exist, for the most part, do not show mongoloids to be particularly abnormal in their metabolic patterns.

The present study is the result of the common observations that as mongoloids age, their skins become rough, dry, and exzematous. Fissures of the lips and corners of the mouth are also frequently seen. These types of lesions are also found in individuals deficient in the B vitamins.

This study is an attempt to determine whether the metabolism of vitamins by mongoloids is abnormal.

METHODS

The subjects used in these experiments were either mongoloids or mentally deficient patients without other obviously abnormal characteristics. During these studies the subjects ate their usual diets in the dining rooms of the Wrentham State School. In the first two studies all food served was weighed and the nutrient intakes calculated from tables of food consumption. The subjects were under continuous supervision to assure complete collections of urine and accurate estimates of food intake.

In the first study, in which subjects aged 10 to 12 years were used, 24-hour urine collections were made for five days. On the sixth day each subject received 2.5 mg thiamine hydrochloride, 2.5 mg riboflavin, 25 mg niacinamide, 50 mg ascorbic acid, 25 mg calcium pantothenate and 2.5 mg pyridoxine hydrochloride. Following the load test two 4-hour urine collections were obtained. In the second study in which older subjects were used, urine was collected every two hours day and night during an experimental period of five days. The purpose of the short collection periods was to study the variation in vitamin excretion. This has been reported in part elsewhere.[1] On the morning of the third day a test dose of 2.5 mg thiamine, 2.5 mg riboflavin, 25 mg niacinamide and 20 mg ascorbic acid was given orally.

In the third experiment, 17 mongoloids and 13 non-mongoloids were given 100 mg of niacinamide at midnight, approximately six hours after their evening meal, and urines were collected during the next six hours. Three weeks later the same groups were given test doses of 5 g of DL-tryptophan at midnight and urines were collected during the next thirty hours. The unsolicited assistance of one of the subjects resulted in the loss of the urine obtained during the six-hour period following the load test.

At various times during these studies urine samples were analyzed for thiamine,[2] riboflavin,[3] niacin,[4] N-methylnicotinamide,[5] xanthurenic acid,[6] creatine,[7] creatinine,[7] and ascorbic acid.[8]

RESULTS

Tables I and II present the results of urine

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TABLE I
Vitamin Intake and Excretion Data, Young Subjects

	Mongoloids				Non-mongoloid				Average	
	1	2	3	4	5	6	7	8	Mongoloids	Non-mongoloids
Age, years	11	12	13	12	12	10	13	11		
Weight, kg	33.6	28.6	28.6	29.0	33.6	27.2	25.0	27.2	30.0	28.3
Height, in.	50	50.5	50	53	55.4	54.2	52	53.7	50.9	53.8
Riboflavin										
Average intake, mg/day	2.9	2.3	2.7	2.3	2.4	2.2	2.4	2.4	2.55	2.35
Average urinary excretion, mg/day	0.65	0.65	1.15	0.96	0.90	0.61	0.92	1.32	0.85	0.94
4 hours after load, total mg	1.35	1.49	0.90	2.15	1.36	0.95	1.91	1.70	1.47	1.48
*Recovery, % of dose	50	55	28	80	49	34	70	63	53	54
Thiamine										
Average intake, mg/day	1.6	1.4	1.5	1.1	1.3	1.5	1.6	1.3	1.40	1.42
Average urinary excretion, mg/day	0.20	0.20	0.25	0.17	0.25	0.38	0.24	0.26	0.21	0.28
4 hours after load, total mg	0.27	0.22	0.17	0.30	0.23	0.08	0.18	0.21	0.24	0.18
*Recovery, % of dose	9.6	7.6	5.2	10.3	7.6	0.8	5.6	6.8	8.2	5.5
Niacin										
Average intake, mg/day	11.3	11.2	9.7	9.6	9.3	11.4	9.9	10.1	10.45	10.17
Average urinary N-Me nicotinamide, mg/day	2.02	2.70	4.31	4.35	3.61	4.70	2.89	3.81	3.34	3.75
4 hours after load, total N-Me nicotinamide, mg	1.10	1.90	1.32	2.96	2.41	3.47	1.47	2.24	1.82	2.40
*Recovery, % of dose	3.2	5.8	2.4	8.9	7.2	10.7	4.0	6.4	5.1	7.1
Average urinary niacin, mg/day	0.32	0.43	0.43	0.46	0.44	0.61	0.29	0.41	0.41	0.44
4 hours after load, total mg	0.16	0.18	0.18	0.24	0.16	0.19	0.21	0.31	0.19	0.22
*Recovery, % of dose	0.44	0.44	0.44	0.64	0.36	0.36	0.64	0.24	0.49	0.40
Ascorbic Acid										
Average intake, mg/day	49	56	47	34	48	54	38	30	47	43
Average urinary excretion, mg/day	5.9	5.8	6.9	6.3	7.5	6.0	6.6	6.7		
4 hours after load test, total mg	1.23	1.13	0.81	2.31	1.72	0.90	1.23	1.09		
*Recovery, % of dose	0.48	0.32	None	2.52	0.96	None	0.28	None		
Creatinine, mg/kg/day	19.2	18.1	19.3	20.6	17.4	19.6	19.4	20.3	19.3	19.2
Creatine, mg/kg/day	2.3	3.3	1.9	1.5	4.5	3.4	2.9	4.7	2.3	3.9

* Excess over the amount expected in the sample as estimated from the previous mean rate of excretion.

TABLE II
Vitamin Intake and Excretion Data, Older Subjects

	Mongoloids		Non-mongoloids	
	1	2	3	4
Age, years	43	20	20	25
Weight, kg	73.6	60.5	70.5	61.4
Height, in.	63.7	58.5	69	65
Riboflavin				
Average intake, mg/day	2.96	2.80	3.09	2.64
Average urinary excretion, mg/day	0.75	0.62	1.14	0.78
4-hour excretion after load, mg	1.46	1.29	1.12	0.80
4-hour excretion, % of dose	53	48	37	67
Thiamine				
Average intake, mg/day	1.74	1.80	1.98	1.72
Average urinary excretion, mg/day	0.38	0.28	0.37	0.14
4-hour excretion after load, mg	0.25	0.39	0.24	0.25
4-hour excretion after load, % of dose	7.16	13.2	7.2	9.2
Niacin				
Average intake, mg/day	19.9	18.1	23.1	20.9
Average urinary N-Me nicotinamide, mg/day	11.71	4.80	10.19	5.46
4-hour urinary N-Me nicotinamide after load, mg	1.59	0.87	2.19	1.75
% of dose	None	0.3	2.0	3.4
Average urinary niacin, mg/day	1.36	0.67	1.24	1.14
4-hour niacin after load, total mg	0.24	0.16	0.24	0.16
4-hour urinary niacin, % of dose	0.05	0.2	0.1	None
Creatinine, mg/kg/day	22.2	24.4	28.5	19.7

analyses made on the subjects of the first two studies prior to the load tests and for four hours after the vitamin supplementation. Nutrient intakes, except for ascorbic acid, approximated or were above recommended allowances[9] for subjects of the ages and sex used. While the ascorbic acid intakes were considerably below the amounts recommended by the Food and Nutrition Board of the N.R.C., they are as high as the amounts considered adequate in Britain[10] and are thus considerably above deficiency levels. The intakes of calories, protein, calcium, phosphorus, iron and vitamin A were also calculated from the food intake data and found to be more than adequate.

There is considerable literature concerned with the excretion of vitamins by subjects fed diverse diets and following load tests varying in amounts and methods of administration. The general methods and findings have been summarized by Unglaub and Goldsmith.[11] Since in this paper we are primarily interested in comparing mongoloids with non-mongoloids, it is not particularly useful to review such material at this time except to point out that, with the exception of ascorbic acid, the excretion values of the subjects used in these experiments reflect the generous nature of the diets fed. The low levels of urinary ascorbic acid before and after the load tests are indicative of the low level of intake of ascorbic acid.

It is apparent from Tables I and II that although considerable individual variation in urinary excretion values was observed, relatively little differences were seen between the mongoloid and non-mongoloid patients. The only apparent differences encountered in these studies were in creatine excretion (Table I) and in N-methylnicotinamide excretion following the load tests (Tables I and II). In both instances excretion values were higher for non-mongoloids than mongoloids. A tendency towards greater excretion of thiamine by mongoloids following the load tests was also observed.

Methylation reactions are involved in the production of N-methylnicotinamide, creatine, and creatinine. The third test in which loads of 100 mg of niacinamide were given was designed to see if there might be a difference in the methylating ability of mongoloids and non-mongoloids. The results presented in Table III show that mongoloids excrete significantly less N-methylnicotinamide and creatinine than non-mongoloids, but not of niacin or xanthurenic acid, following a load test of niacinamide.

TABLE III

The Effect of Niacinamide on Urinary Metabolites

	Mongoloids	Non-mongoloids
Number of subjects	16	13
Urine volume, ml	238 ± 28	302 ± 29
Creatinine, mg	247 ± 18	347 ± 30 $p < .05$
N-Methylnicotinamide, mg	12.9 ± 1.3	19.1 ± 0.7 $p < .005$
Xanthurenic acid, mg	6.7 ± 1.1	7.2 ± 0.4
Niacin, γ	294 ± 27	371 ± 68

Figures include standard error of the mean.

The relationship between niacin and tryptophan metabolism led to the fourth study in which each subject received 5 g of DL-tryptophan. The results shown in Table IV do not show a statistically significant difference in the excretion of creatinine and N-methylnicotinamide by mongoloids and non-mongoloids following a tryptophan load test although more of these metabolites were excreted by the non-mongoloids. In this study the excretion of xanthurenic acid was significantly ($p = 0.05$) greater in non-mongoloids than in mongoloids.

TABLE IV

Effect of DL-Tryptophan on Urinary Metabolites

	Mongoloids	Non-mongoloids
Number of subjects	17	13
Urine volume, ml	1239 ± 46	1166 ± 156
Creatinine, mg	830 ± 44	916 ± 27
N-Methylnicotinamide, mg	11.2 ± 2.9	13.1 ± 1.5
Xanthurenic acid, mg	26.2 ± 2.3	38.4 ± 5.6

Figures include standard error of the mean.

DISCUSSION

Since no other explanation is available, it would seem certain that metabolic changes must underlie the marked abnormalities which occur in mongolism. Those metabolic changes which result in epithelial abnormality may be relatively specific for the tissue affected and at

the tissue level, or they may be more general in nature and contribute to other phases of the over-all picture of mongolism. Although the present studies do not indicate any difference in the way that riboflavin is handled by mongoloids and non-mongoloids and the differences observed in thiamine excretion are not marked, it cannot be concluded that the requirements and metabolism of these two vitamins are the same for mongoloids and non-mongoloids. Vitamin excretion tests are relatively non-critical particularly when intakes are high. For the purposes of these studies more limited vitamin intakes would have been advantageous.

The difference in excretion of N-methylnicotinamide following niacinamide administration is interesting. It is impossible at this time to evaluate the significance of this observation with regard to the pathology of mongolism. It may be unrelated to the physical manifestations of the disease or may represent a basic difference in the metabolism of niacin related to the epithelial and other abnormalities of mongolism.

It is generally considered that creatinine excretion is more closely correlated with active muscle mass than with body weight. The weights of the large groups of subjects used in the third and fourth studies were essentially the same averaging with standard deviations 53.4 ± 7.6 kilos for the mongoloids and 53.7 ± 6.8 kilos for the non-mongoloids. Although mongoloids are generally thought to be more obese than non-mongoloids, the creatinine coefficients (mg of creatinine/kilo body weight/24 hr) of the two groups obtained from 24-hour control urine collections were not significantly different statistically, being with standard errors of the mean 23.7 ± 1.8 mg for the non-mongoloids and 20.2 ± 1.1 mg for the mongoloids. Nevertheless these values which are in the normal range may represent the trend towards increased obesity in the mongoloids. During the six hours following the 100 mg niacinamide load test, the differences in creatinine excretion of the two groups were increased to statistical significance (Table III). The lower excretion of both N-methylnicotinamide and creatinine by mongoloids following the niacinamide load test suggest the possibility of a defect in the methylating ability of mongoloids since both these compounds are end products of methylation reactions. It is possible that the lowered creatinine values in the mongoloids reflect a low B.M.R. and that the decreased N-methylnicotinamide excretion of mongoloids is indicative of a generally limited metabolic capacity. However, the B.M.R. of mongoloids has been reported normal[12] or only slightly reduced.[13,14] Furthermore, serum protein-bound iodine levels between mongoloid children and controls of the same age have been found to be similar.[15]

The significance of the difference in xanthurenic acid excretion following tryptophan administration (Table IV) needs further study. Usually in tests of this kind two times as much tryptophan are used as in this experiment. In view of the recent interest in the role of tryptophan metabolism in mental disease,[16] extension of these studies to include other tryptophan metabolites would appear worthwhile.

SUMMARY

The metabolism of a number of water-soluble vitamins by mongoloid and non-mongoloid, all mentally deficient children, has been studied. Relatively little differences in the excretion of thiamine, riboflavin, niacin, N-methylnicotinamide and vitamin C were observed prior to vitamin load tests. Following the load tests no significant difference was observed in the excretion of riboflavin by mongoloids and non-mongoloids and the excretion of thiamine although slightly greater in mongoloids was not marked. The administration of 100 mg of nicotinamide resulted in significantly less excretion of N-methylnicotinamide and creatinine by mongoloids than non-mongoloids. The possibility of a defect in the methylating ability of mongoloids has been suggested. Following the feeding of 5 g of DL-tryptophan, significant differences in N-methylnicotinamide and creatinine excretion were not observed, but the excretion of xanthurenic acid was lower in mongoloids than non-mongoloids.

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Lean-Body Mass Creatinine-Coefficient Deficit and Urinary Steroids

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THE PROBLEM of determining the degree of protein depletion in individuals of advanced age or following periods of chronic debilitation is probably the most basic in the clinical management of such patients. Nitrogen balance studies are impractical and often unrewarding—especially when changes in nitrogen metabolism take place slowly.

A great need exists therefore for the establishment of a practical technic for the determination of the status of nitrogen equilibrium. One of the proposed technics which is under investigation in this laboratory will be reported here.

The quantity of creatinine excreted in the urine in 24 hours is usually considered to be a function of muscle mass. Although the constancy of this quantity is not absolute, within wide ranges it is usually a function of the weight of the individual. Thus the term *creatinine coefficient* refers to milligrams of creatinine per kilogram of body weight. Aside from a variety of pathologic events, the most important variables which reduce the utility of the creatinine coefficient are those of fat and water contents.

Behnke, Osserman, and Welham[1] derived the following approximate equation for estimating the lean-body-mass (L.B.M.) for young men who were not athletes:

$$\text{L.B.M. (g)} = 2 \times (\text{Ht. [cm.]})^2 \qquad (1)$$

With the use of this equation, it is possible to calculate a L.B.M. creatinine-coefficient, thus:

$$\frac{\text{24-hour creatinine value}}{\text{L.B.M. (kilo)}} = \text{C.C.}_{\text{L.B.M.}} \qquad (2)$$

$\text{C.C.}_{\text{L.B.M.}}$ should be a much more reliable index of muscle mass than the usual creatinine-coefficient. If now a mean $\text{C.C.}_{\text{L.B.M.}}$ is established, deviation from this value should reflect a loss in muscle mass, thus:

$$\text{C.C.}_{\text{L.B.M.}} \text{ observed} - \overline{\text{C.C.}}^*_{\text{L.B.M.}} = -\Delta\text{C.C.} \qquad (3)$$

Another feature of this investigation pertains to the well known fact that with aging the excretion of ketosteroids and androgens diminish markedly while that of the corticoids is less affected.[2] This would lead to the expectation that the antianabolic† influence of the gonadal-adrenal axis might increase. Certain evidence suggests that illness advances the imbalance in the production of these steroids.[3,4] The metabolic consequences of these changes should therefore be observed sooner in individuals who are chronically ill.

A study was therefore made of $\text{C.C.}_{\text{L.B.M.}}$ and the urinary ketosteroids and corticoids in individuals who were well and those who were chronically ill.

METHODS

In an attempt to reduce disuniformity of population selection, the patient load was drawn mainly from the Veterans Administration Center and consisted of males from 23 to 80

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* The line over the C.C. signifies the mean value.

† Antianabolic is used here in the sense that it describes a category of events which are opposite to the anabolic. It should not be implied that the author has taken sides on the issue of whether the disturbances in nitrogen metabolism are related to antianabolic or catabolic influences.

years of age. Random complete 24-hour collections of urine were made without previous preparation of the patient. The urine was refrigerated during collection. No preservatives were added. Creatinine and hormonal determinations were made as soon as possible and the urines were refrigerated until this was done. Creatinine was determined by the procedure of Bonsnes and Taussky,[5] ketosteroids by the procedure of Drekter *et al.*[6] and corticoids by the method of Porter and Silber.[7]

The control group of 36 men was chosen from staff members, associates, other workers, and patients with minor dermatologic ailments.

Patients with clinical evidence of muscular, renal or hepatic disease were excluded from this study. In addition, individuals whose height could not be correctly measured due to deformity or bone disease were not included, nor were patients with acute illnesses accepted. The major causes of illness were as follows: 18, various manifestations of atherosclerosis and its sequalae; 9, chronic cutaneous disease; 8, chronic pulmonary disease; 2, valvular disease; 3, gastrointestinal ulceration; 4, neurologic diseases; 3, psychologic disorders; 5, hypertension; 12, arthritis; 2, resections for cancer; 1, a blood dyscrasia. These included individuals with multiple illnesses. The total number was 51 patients.

RESULTS

The mean $C.C._{L.B.M.}$ for 36 normal men was 27.1. All values of 27.0 or above were assigned to the group with $-$C.C. of 0 (Table I). This category included 21 men. The mean $-\Delta$C.C. of the remaining 15 normal subjects was 4.6. This group was then subclassified into individuals above or below this average. Thus, nine men had an average $-\Delta$C.C. of 2.0 and six men, four of them over 60 years of age, had an average $-\Delta$C.C. of 7.4. The significance of these ratios will be discussed below.

TABLE I

Lean Body Mass Creatinine Coefficient Deficient ($-\Delta$C.C.) and Urinary Ketosteroids (K) and Corticoids (C)

Number	Age		$-\Delta$C.C.	K *mg*	C *mg*	K/C	K^2/C
	Range	Mean					
36 normal male adults							
21	23–72	44	0	13.8 ± 0.9*	8.9 ± 0.6	1.62 ± 0.10	22.9 ± 2.2
6	35–77	58	2.0 ± 0.3	11.5 ± 1.0	6.7 ± 0.5 (P = <0.02)	1.75 ± 0.15	20.5 ± 0.5
6	44–77	62	8.4 ± 0.4	7.9 ± 1.0 (P = <0.01)	5.7 ± 0.6 (P = <0.01)	1.39 ± 0.09	10.3 ± 1.4 (P = <0.01)
51 chronically ill males							
25	29–80	54	0	13.2 ± 0.7	9.8 ± 0.6	1.43 ± 0.09	19.1 ± 1.8
11	40–70	52	3.3 ± 0.1	11.6 ± 1.1	8.3 ± 1.0	1.48 ± 0.13	18.0 ± 3.1
15	42–71	59	8.4 ± 0.7	8.3 ± 0.8 (P = <0.01)	6.3 ± 0.4 (P = <0.01)	1.32 ± 0.11 (P = <0.02)	11.9 ± 2.1 (P = <0.01)

All data based on 24-hour values. Only statistically significant P values are given.
* Standard error.

The chronically ill group was similarly classified. Twenty-five patients had a $-\Delta$C.C. of 0. In 26 $-\Delta$C.C. averaged 6.3; 11 had values less than this and 15 exceeded it. The average of the former group was 3.3, the latter 8.4. Thus 29 per cent of the chronically ill individuals exhibited large $-\Delta$C.C. values in contrast to 17 per cent of the well subjects.

Table I reveals that statistically significant differences in urinary ketosteroids and corticoids were found in the group which deviated the most from the mean lean body mass creatinine coefficient, i.e., with the greatest $-\Delta$C.C

This held true for both the chronically ill and the control groups. The possible significance of this observation is discussed forthwith.

DISCUSSION

The limitations in the use of creatinine excretion as a function of muscle mass are quite well known. These include the presence of renal, hepatic, and muscular disease. Furthermore, constancy of excretion is not absolute. Perhaps the most important reason for the failure to put creatinine coefficient into more frequent use in clinical medicine is related to the absence of a device heretofore for measuring lean body mass. The equation of Behnke, Osserman, and Welham[1] represents an important step forward in this direction. If the early observations reported here are confirmed, the determination of $-\Delta$C.C., with suitable refinements, may prove to be useful in estimating degree of protein depletion, in evaluating the influence of anabolic steroids, and in nutritional management.

The use of urinary steroid determinations in the evaluation of metabolic disturbances induced by the mixture of endogenous steroids produced by the adrenals and gonads involves the usual suppositions. It must be assumed that the urinary excretion reflects the situation in the circulation, that end-organ sensitivity is similar from individual to individual, and that the biologic activity of the mixture of steroids as it occurs in the blood may be known from a knowledge of their individual activities. The steroids were not subfractionated and consequently the findings may have a limited utility in attempts to evaluate the biologic activity of these materials or their precursors in regard to the antianabolic-anabolic influence exerted by them. Nevertheless in a broad sense the decrease in urinary 17-ketosteroids reflects decreases in the production of androgenic and therefore anabolic precursors.

The K/C ratio attempts to show the disturbance in the relative amounts of anabolic steroids to the antianabolic corticoids. It also should be interpreted to represent the activity of 1 mg of "androgens" in the presence of corticoids. The ratio K^2/C was contrived in an attempt to represent the 24-hour equivalence of the activity of the "androgens." Thus since K/C represented the activity of 1 mg of "androgens," $K \times K/C$ would represent the 24-hour value. The finding that statistically significant differences occur would seem to make the use of this ratio justifiable.

Investigations in the field of aging which have been carried out in this laboratory emanate from the hypothesis that with aging, such disturbances in gonadal-adrenal-steroid production occur that increasing antianabolic influences are exerted, and that this phenomenon is accelerated by "usage."[4] The ensuing changes seem to advance certain other aspects of aging such as those related to the connective tissue.[8]

The observation that large $-\Delta$ C.C. values occur more frequently in persons who have been chronically ill and that K, K/C, and K^2/C values are lower in those individuals is consistent with the concept stated above. However, the possible coincidental occurrence of two age-associated phenomena, consequent to more fundamental aging processes must not be overlooked.

SUMMARY

The Lean Body Mass Creatinine Coefficient ($-\Delta$C.C.) is proposed as a device for evaluating nitrogen deficits in individuals who are free of renal, hepatic, and muscular disease and whose correct height is determinable. In a group of men with a variety of chronic illness, large $-\Delta$C.C. values were observed in 29 per cent of the cases in contrast to a similar group of normal men in whom large deficits occurred in only 17 per cent. A relevant finding was the presence of an increased antianabolic influence as determined by urinary steroid studies in patients with large $-\Delta$C.C. values.

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"Saturation" Studies with Vitamin B_{12} in Human Subjects

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DETERMINATION of the amount of vitamin B_{12} excreted in the urine after parenteral administration of a single test dose of this substance has not proved to be of value in distinguishing between normal subjects and patients with pernicious anemia or related megaloblastic anemias. After repeated doses of vitamin B_{12}, given either parenterally or orally, the percentage of the amount administered that is excreted in the urine increases, as do serum concentrations of both free and bound vitamin, suggesting that saturation of body stores may occur.[1–5]

It seemed possible that in the course of saturation of tissue stores of vitamin B_{12} by repeated parenteral injections of this substance, measurement of urinary excretion and of serum levels of free and bound vitamin might indicate abnormalities of metabolism in vitamin B_{12} deficiency states. In addition, the length of time required to attain maximum serum concentrations and maximum urinary excretion of the vitamin might reflect the degree of depletion of tissue stores. The maximum binding capacity of serum proteins for vitamin B_{12} might be estimated *in vivo* by provision of an excess of free vitamin after tissue stores had been saturated. The relationship of free and bound vitamin B_{12} in the serum to urinary excretion of this substance could be studied at various stages of the saturation procedure.

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MATERIALS AND METHODS

Six normal subjects, five patients with pernicious anemia in relapse, five patients with other types of megaloblastic anemia responsive to vitamin B_{12} therapy, and five patients with diabetes mellitus were selected for this study. The patients with diabetes were included because of reports in the literature[6] which suggested abnormalities of vitamin B_{12} requirement or utilization in this condition. All subjects were hospitalized in a metabolism ward throughout the period of observation. The patients with diabetes were maintained in a satisfactory state of control by administration of suitable diets and insulin. All other subjects were given standard hospital diets.

After one to three days of control observations, each subject was given 50 μg of crystalline vitamin B_{12} intramuscularly daily for ten days. This amount was selected in order to facilitate comparison of data with those previously obtained using this dosage. Consecutive 24-hour urine samples were collected during this period for measurement of total vitamin B_{12} content. Blood samples were obtained prior to the initial injection, while subjects were in the post-absorptive state, and at intervals of 1, 4, 8, and 24 hours thereafter. Additional blood samples were obtained at intervals of one to four days.

At the conclusion of the initial ten-day period, the dose of vitamin B_{12} was increased to 1,000 μg daily, intramuscularly, for a second period of ten days. No urine collections were made during this time, but blood samples were collected as in the initial period.

No vitamin B_{12} was administered on the 21st, 22nd, or 23rd days of the experimental period to permit urinary concentrations of the vitamin to return to their pre-injection levels. On the 24th day, blood was obtained for determination of vitamin B_{12} concentrations and a single injection of 50 μg of the vitamin was given intramuscularly. Urine was collected during the subsequent 24-hour period, and additional blood samples were taken at 1, 4, 8, and 24 hours after administration of the test dose. One patient with non-Addisonian megaloblastic anemia received 3,000 μg of crystalline vitamin B_{12} orally in lieu of the final intramuscular injection.

Serum was separated promptly and if not analyzed for total and bound vitamin B_{12} immediately was kept frozen at 4°C until analysis could be carried out. Aliquots of the twenty-four hour urine samples were adjusted to pH 6.8 and kept frozen until analyzed.

Microbiologic assay of vitamin B_{12} activity in urine, employing *Lactobacillus leichmannii* (*ATCC 4797*), was carried out according to the method of Thompson,[7] as modified by Register and Sarett.[8] Total vitamin B_{12} activity in serum was measured by the method of Rosenthal and Sarett,[9] and bound vitamin B_{12} activity by the method of Miller.[10] Free vitamin B_{12} activity was calculated as the difference between total and bound vitamin B_{12} activity.

RESULTS

All patients with anemia responded satisfactorily to the administration of vitamin B_{12} as determined by increases in reticulocytes[11] and in erythrocyte counts.[12]

The patterns of urinary excretion of vitamin B_{12} in normal subjects and in patients with pernicious anemia during the first ten days of the "saturation" regimen and in response to the final intramuscular test dose on the 24th day are shown in Fig. 1. Average urinary excretion of the vitamin following the first injection of 50μg was essentially the same for the two groups, approximating 12 to 15 per cent of the dose administered. Wide variation in excretion within each group is apparent from the range of values found, although it was less pronounced in the patients with pernicious anemia than in the normal subjects.

In both groups, the amount of vitamin B_{12} excreted in the urine increased progressively for the first three days of the regime. By the fourth day, urinary excretion of the vitamin

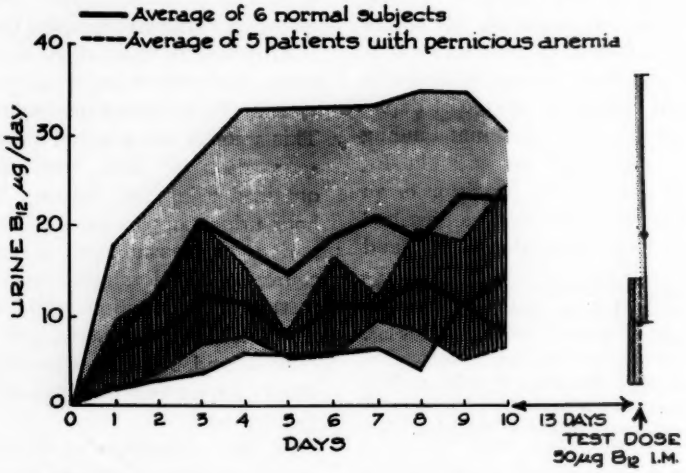

Fig. 1. Urinary excretion of vitamin B_{12} by normal subjects and by patients with pernicious anemia. Range of individual values for normal subjects indicated by stippled area, for pernicious anemia patients by cross-hatched area.

reached average values of approximately 40 per cent of the dose administered in the normal subjects and 20 per cent in the patients with pernicious anemia. From the fourth to the tenth days the average amount of the vitamin excreted in the urine showed no further systematic increase in either group. Wide variations in consecutive daily values were observed in individuals of both groups. Although average urinary excretion of vitamin B_{12} after the third day was definitely lower in the patients with pernicious anemia than average excretion in the normal subjects, values below the normal range were observed on only two occasions.

Following the final test dose of 50 μg on the 24th day of the regimen, average urinary excretion of vitamin B_{12} was essentially the same as that found for each group after the third day of the injections. The amount of vitamin B_{12} found in the urine of one patient with pernicious anemia was well below the range of values found in normal subjects.

The pattern of urinary excretion of vitamin B_{12} during the saturation period of five patients with diabetes mellitus did not differ significantly from that of the normal subjects (Fig. 2). The average amount of the vitamin excreted by this group after the final 50 μg test dose was approximately 50 per cent greater than that observed in the normal subjects, although all individual values fell within the normal range.

Urinary excretion studies were completed in four of the five patients with megaloblastic anemia other than pernicious anemia. In two of these, the excretion of vitamin B_{12} during the first ten days of the saturation period approximated average values of normal subjects. In the third patient, values resembled those of the pernicious anemia group, while those of the fourth subject fell midway between the average values for the normal subjects and the pernicious anemia patients. In the three subjects who received an intramuscular test dose of 50 μg of vitamin B_{12} on the 24th day, the amount of the vitamin excreted in the subsequent 24-hours was not significantly different from the average amount excreted by these individuals during the third to the tenth day of the regimen. The patient who received the oral test dose of 3,000 μg of the vitamin excreted less than 0.4 μg of vitamin B_{12} in the next 24 hours.

A significant increase in the concentration of both free and bound vitamin B_{12} in serum was observed in all subjects during the saturation period. Increases in bound vitamin B_{12} were most rapid and pronounced in the normal subjects and in the patients with diabetes (Fig. 3). The average maximum concentration of bound vitamin B_{12} in patients with megaloblastic anemia was significantly lower than that of normal or of diabetic subjects. Patients H. C. and A. B., however, attained levels of bound vitamin B_{12} comparable to those observed in the normal group.

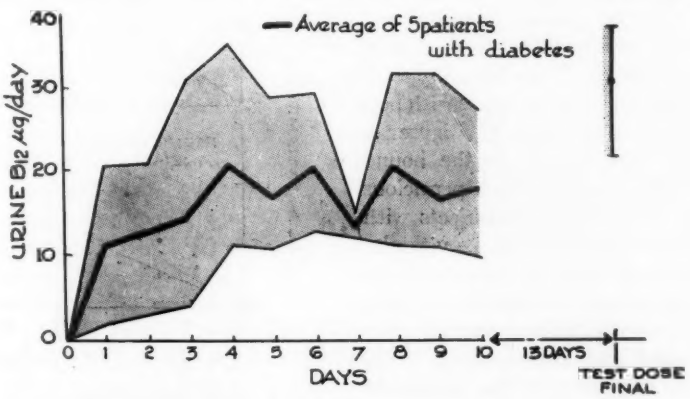

Fig. 2. Urinary excretion of vitamin B_{12} by patients with diabetes mellitus. Range of individual values indicated by stippled area.

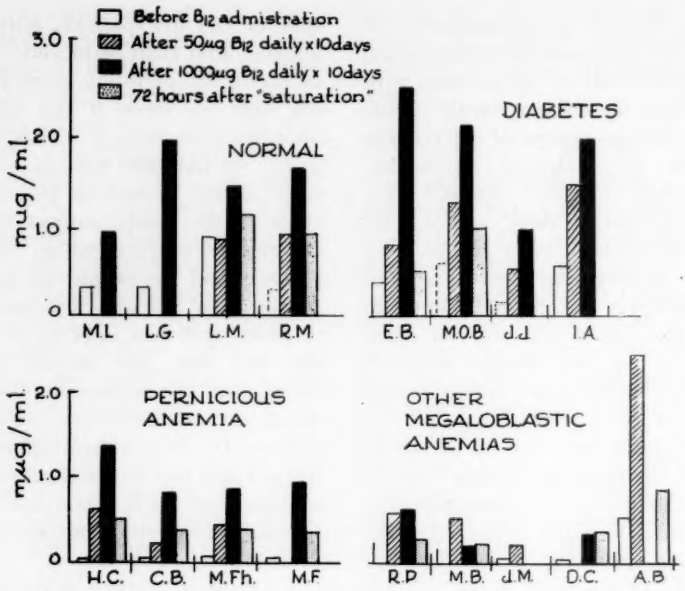

Fig. 3. Changes in concentration of bound vitamin B_{12} in serum during "saturation" regimen.

Total vitamin B_{12} concentrations in the serum of patients with megaloblastic anemia were lower at the end of the saturation period than those of the normal or the diabetic subjects. Since initial concentrations of total and bound vitamin B_{12} in the serum of the anemia patients were lower than those of the other groups, it was necessary to determine whether the amount of vitamin B_{12} bound to serum protein was dependent upon the total amount of the vitamin present in the serum. The relationship between the concentration of bound and total vitamin B_{12} in all samples of serum of normal subjects and patients with pernicious anemia is illustrated in Figure 4. Less vitamin B_{12} was present in the bound form in the serum of patients with pernicious anemia than in that of normal subjects with equivalent total concentrations of the vitamin. The difference in regression coefficients of the two groups is significant at the 1 per cent level. Much more variability in the relationship between total and bound vitamin B_{12} concentrations is apparent in the pernicious anemia patients than in the normal subjects. When data obtained from the patients with non-Addisonian megaloblastic anemia are analyzed in like fashion, a regression line similar to that of the pernicious anemia group is

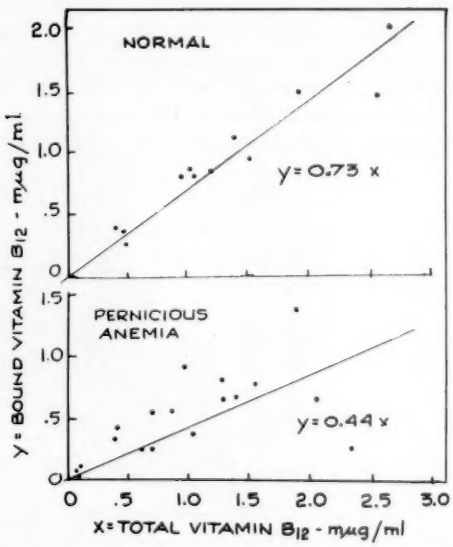

Fig. 4. Relationship between total and bound vitamin B_{12} in the serum of normal subjects and of patients with pernicious anemia during "saturation" regimen.

obtained ($y = 0.51x$). This also differs significantly at the 1 per cent level from that of the normal subjects.

No correlation could be demonstrated between the amount of free, bound, or total vitamin B_{12} present in the serum during the 24-hour periods following administration of either the initial or the final test dose of the vitamin and the amount of this substance excreted in the urine during the same periods. This was determined by plotting concentrations of each of these forms of the vitamin at 1, 4, 8, and 24 hours following the injections, connecting the points by straight lines, and measuring planimetrically the areas so defined. The values thus derived were then plotted against the amount of vitamin B_{12} excreted in the urine during the same period. In the case of M. B., who received 3,000 μg of vitamin B_{12} orally as the final test dose, only 0.38 μg of the vitamin was found in the subsequent twenty-four hour urine sample, despite an increase of free and bound vitamin B_{12} in the serum which exceeded that of some patients who excreted from 2.3 to 14.7 μg following the intramuscular test dose.

DISCUSSION

The marked variability of urinary excretion of vitamin B_{12} among the groups of subjects studied, as well as in individual subjects from day to day, during a constant regimen of vitamin B_{12} injections was noteworthy. These findings would appear to preclude the use of urinary excretion after single or repeated injections of vitamin B_{12} as a measure of body stores of this substance. Although average values for urinary excretion of vitamin B_{12} in patients with megaloblastic anemia were lower than those of the normal and diabetic subjects individual values remained in the low normal range in most instances.

Such results might be due to continued storage of the vitamin. If this is the case, our observations indicate that administration of 10,500 μg of vitamin B_{12} in a period of 23 days is not sufficient to replenish body stores of this substance. If it is assumed that 50 per cent of the vitamin is retained during the ten days of injections of 50 μg daily, and 10 per cent is retained during the period of injections of 1,000 μg daily, this regimen would furnish 1,275 μg of vitamin B_{12} for replenishment of body stores. This is considerably less than the amount normally present in the tissues of nonanemic subjects, as estimated from analyses of Girdwood.[13]

Other possible explanations for the low average urinary excretion of vitamin B_{12} by patients with pernicious anemia during this regimen are (1) excretion of the vitamin by extra-renal routes, (2) excretion in the urine in a form not detectable by the microbiologic assay used and (3) increased destruction of vitamin B_{12} in the body. Studies with vitamin B_{12} labeled with radioactive Co^{60}, to be published elsewhere, do not support the first two of these possibilities and indicate that if increased destruction of the vitamin does occur, the cobalt of the molecule is retained in the body.[14]

An interesting finding of this study is the difference between concentrations of bound vitamin B_{12} attained in the serum of normal subjects during the regimen and those attained in serum of patients with megaloblastic anemia. The failure of serum of the anemia group to bind the vitamin to as great an extent as did that of the normal or of the diabetic subjects might be due to deficiency of the factor in serum which combines with the vitamin. Studies in our laboratory in which this serum factor was estimated by an *in vitro* technic[15] lend support to this concept. Another possible explanation, which has been suggested previously by other investigators,[16,17] is that intrinsic factor may possess activity beyond that of enhancing absorption of vitamin B_{12} from the gastrointestinal tract or that there may be an "extragastric intrinsic factor." Miller[15,18] has found that intrinsic factor promotes the combining of vitamin B_{12} to certain serum proteins and increases the uptake of vitamin B_{12} by tissues *in vitro*. Intrinsic factor may have similar functions *in vivo*. This explanation would appear to be more applicable to findings in patients with pernicious anemia than in those with non-Addisonian megaloblastic anemia. However, we have reported studies[19] made with an *in vitro* technic that suggest that some patients with non-Addisonian megaloblastic

anemia, as diagnosed by the Schilling test,[20] may have varying degrees of deficiency of intrinsic factor in their gastric secretions.

Mollin and Ross[2] reported that urinary excretion of vitamin B_{12} after parenteral administration of this substance is proportional to the concentration of the free vitamin in the serum. Our studies have shown no relationship between the concentration of free vitamin B_{12} in serum and urinary excretion of the vitamin, either in normal subjects or in those with megaloblastic anemia or diabetes. This discrepancy in findings presumably is related to methodology. Mollin and Ross[21] measured free vitamin B_{12} directly after heating serum to 56° C for 30 minutes and total vitamin B_{12} after heating to 100° for 15 minutes. *Euglena gracilis, varo bacillaris* being used as the test organism. Bound vitamin B_{12} was determined by difference. In the technic used by us, bound vitamin B_{12} is determined directly after adsorption of the free vitamin on charcoal, free vitamin B_{12} being determined by the difference between total and bound B_{12}. *L. leichmannii* is used as the test organism. The difference in findings with the two technics could be explained also by postulating the presence of a third form of vitamin B_{12} in serum which is loosely bound to protein, is adsorbable on charcoal and is not excreted by the kidneys.

SUMMARY AND CONCLUSIONS

Normal subjects and patients with megaloblastic anemia or diabetes mellitus were studied during a "saturation" regimen of parenterally administered vitamin B_{12}. Urinary excretion and concentrations of free and bound vitamin B_{12} in serum were determined. Average values for urinary excretion of the vitamin in patients with megaloblastic anemia were lower than those of normal or diabetic subjects, although individual values were within the normal range in most instances. It seems likely that the dosage of vitamin B_{12} employed during this regimen, although large, was not sufficient to produce saturation of body stores in the severely depleted subject. The marked variability in urinary excretion of the vitamin during a constant regimen appears to preclude the use of urinary excretion tests after single or repeated injections of vitamin B_{12} as a measure of body stores. No correlation could be demonstrated between the amount of free vitamin B_{12} in the serum during a 24 hour period following a test dose of this substance and the amount of the vitamin excreted in the urine during the same period. The discrepancy between these results and those reported by other investigators is presumably related to differences in methodology.

Levels of bound vitamin B_{12} attained in the serum of patients with megaloblastic anemia during the "saturation" regimen were lower than those observed in the serum of normal or diabetic subjects. In serum of normal subjects, 73 per cent of the total vitamin B_{12} was present in the bound form, while only 44 to 51 per cent was bound in the serum of patients with megaloblastic anemia. These observations may indicate deficiency of the protein factor in serum which combines with vitamin B_{12} or deficiency of some other substance, such as intrinsic factor, which promotes the combining of the vitamin with serum protein in patients with megaloblastic anemia.

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Caloric Equivalents of Gained or Lost Weight

MAX WISHNOFSKY, M.D.

WHAT is the caloric equivalent of one pound of body weight, gained or lost? To put the question in other words: How many calories in excess of the amount necessary to maintain caloric equilibrium will produce a gain of one pound of body weight; conversely, what caloric deficit will determine a loss of one pound of body weight? It is strange that a marked diversity of opinion exists among authorities on this subject. In this article an attempt will be made to clarify the matter.

PHYSIOLOGIC PRINCIPLES

It was shown by Bozenraad[1] that the average fat content of human adipose tissue taken from various parts of the bodies of well-nourished subjects is 87 per cent. One pound (454 g) of human adipose tissue, therefore, contains 395 g of fat. The caloric value of one g of animal fat is 9.5; consequently, the caloric equivalent of one pound of human adipose tissue may be considered to be about 3,750 cal.

Leathes[2] pointed out that carbohydrate and protein cannot be stored dry, but retain with them three or more parts of water, while fat can be stored in an almost pure state. Retention of a gram of fat gives the body 9 cal of reserve energy, while retention of a gram of water-soaked glycogen or protein provides only about one calorie. Failure to recognize this fundamental point has resulted in the greatest bedevilment.

What is the metabolism of carbohydrate, protein and fat in individuals in negative or positive caloric balances? The caloric value of the glycogen stores in the human body, as compared to protein and fat, is insignificant and undergoes but trivial changes when the individual is in clinical negative or positive caloric balance.

With regard to protein metabolism, the following may be noted. Strang *et al.*[3] studied 13 obese patients under the especially rigid conditions of the metabolic service for an average period of 59 days. The average diet provided 58 g of protein, 14 g of carbohydrate, and 8 g of fat, with a total value of 360 cal. They state: "During the first three weeks of dieting, patients may lose nitrogen to the extent of 40–50 per cent above the nitrogen intake. The addition of 10 to 15 g of carbohydrate to the diet causes an abrupt drop in the nitrogen output and the attainment of a permanent nitrogen balance without the alteration of the nitrogen intake." Another group of patients studied by Strang and Evans[4] was kept approximately in nitrogen equilibrium on diets of 600 to 650 cal.

There is a paucity of work on indirect methods for the study of body composition and the findings are conflicting. Behnke, Osserman, and Welham,[6] in a study of "lean body mass," found: (1) On a restricted diet accompanied by body weight loss constancy in the weight of the lean body mass indicates that nitrogen balance is maintained and that the "lost" tissue is fat. (2) In individuals who gained weight, the changes in the lean body mass were slight and the calculated densities of the tissues gained were all within the range of body fat. Keys, Anderson, and Brožek,[5] in a study of weight gain from simple overeating made observations which are at variance with those of Behnke *et al.*[5] They found the tissue mass gained to be 13 to 15 per cent extracellular fluid, 61 to 64 per cent fat, 0 to 1 per cent glycogen, and the remainder protein.

More recently Young *et al.*[7] studied the nitrogen balance in eight obese young men during an eight-week reducing period during which the protein intake was 115 g daily and the mean weight loss 22.6 pounds. The minimum protein requirement when an individual is in caloric balance is between 0.5 and 0.7 g/kg/body weight/day, so that these subjects re-

ceived about twice the minimum requirement. During this eight-week reducing period, four of the subjects were in nitrogen equilibrium, and four in slight negative nitrogen balance.

The conclusions that may be drawn are as follows: (1) Glycogen changes are insignificant when a patient is in negative or positive caloric balance. (2) On a customary low-calorie reducing diet, where the protein intake is high, the patient is either in nitrogen equilibrium or in slightly negative caloric balance. (3) Under the circumstances, the caloric deficit in weight reduction is primarily made up by the catabolism of fat.

In total fasting the metabolism is entirely different. In contradistinction to the obese individual on a low-calorie diet who is practically in protein and carbohydrate (glycogen) equilibrium, here decided protein and carbohydrate deficits occur. The loss of one pound (454 g) of protein will yield about 1,850 cal. There will be a concomitant loss of over 3 pounds of water. The loss of 4 pounds of body weight when 1 pound of protein is catabolized, will result from an expenditure of only 1,850 cal; whereas an individual on a low-calorie diet (negative calorie balance) but in protein equilibrium will lose only one-half pound from the same caloric expenditure. When carbohydrate (glycogen) is catabolized the same obtains as with protein. However, since the amount is comparatively small, for practical purposes it need not be considered.

CRITICAL ANALYSIS AND EVALUATION OF THE PERTINENT LITERATURE

Slonim[8] states: "If a person eats more than 35 cal/kg of his ideal weight per day, he will gain weight at the rate of 1 g of fat plus 1 g of water for each 9 cal in excess. If he eats less than the ideal amount, he will lose correspondingly."

According to this concept 1 g of body weight gained or lost, has a value of $4^1/_2$ cal; 454 g (1 lb) will therefore have a value of 2,042 cal. The error of this concept is the idea that a gram of water is stored with each gram of fat. As was pointed out by Leathes,[2] fat is stored "dry" with a negligible amount of water. Further proof of this will be strikingly demonstrated when the work of Benedict is considered below. It may be noted that from the teleologic standpoint it is vital for the very survival of the organism to have a very concentrated source of energy to act as a reserve during a scarcity of food. Thus, adipose tissue, with a caloric value of 8.3 per g, furnishes in an ideal manner.

Strang, McCluggage and Evans[3] made a careful study of the influence of low calorie diets on weight reduction in obese persons. Thirteen persons were placed on a daily diet of 360 cal for a period of 59 days during which time the average weight loss was 0.6 pound per day. They estimated the number of calories necessary to maintain caloric equilibrium in these patients to be 2,500 per day, so that the 0.6 pounds of body weight lost had an equivalent of 2,100 cal. One pound would therefore have a caloric value of 3,500. This is in striking agreement with the value of 3,700 cal obtained by the determination of the caloric value of one pound of human adipose tissue (Bozenraad).

The estimated figure of 2,500 cal to maintain caloric equilibrium decreases slowly with loss of weight and would result in a slight reduction in the caloric equivalent of one pound of body weight lost. This is compensated for by the fact that during the first two to three weeks, as a result of a negative nitrogen balance, there were losses of water. If these losses of water had not occurred, the caloric equivalent of one pound of body weight lost would be higher than 3,500. The two factors apparently neutralize each other.

Joslin,[9] states: "The caloric value of 1 kg of body weight is problematical but most important. Benedict's[10] normal subject for the last 27 days of his fast of 31 days lost an average of 0.7 per cent of his body weight daily, the equivalent of 3,258 cal/kg or approximately 1,500 cal/lb/body weight. For comparison Joslin[11] had an experiment with a healthy nurse who in 1916 volunteered to go through the exact procedure to which diabetics were subjected that time. Her loss of weight in 20 days represented the equivalent of 3,170 cal for each kilogram of her body lost, and this agrees quite closely with Benedict's fasting subject. There-

fore, one must assume that a patient will not lose, or conversely, gain a pound of actual body tissue unless he receives at least 1,500 cal less or more food than he requires for his standard metabolic need, computed for rest and exercise."

In his conclusions Joslin makes the error of failing to differentiate between the metabolism of the individual who is on a low-calorie diet but who is in protein and carbohydrate (glycogen) equilibrium, and one who is fasting and has marked protein and glycogen deficits. The significance of this point is strikingly demonstrated in Benedict's observation.

Benedict's subject fasted for 31 days, partaking only of distilled water. The pertinent findings are best expressed in Benedict's own words.[12] "During the 31-day fast this subject actually excreted 277.32 g of nitrogen in the urine, thus averaging 8.95 g of nitrogen per day. This would correspond to 1,664 g (3.7 lb) of protein or 8,319 g (18.3 lb) of flesh. Since the entire loss of body weight of this subject was 13.25 kg (30 lb), it can be seen that 63 per cent of the total loss may be accounted in flesh catabolized." Study of Table 63, page 412,[12] shows that whereas during the 31-day fast, the amount of water lost concomitantly from the catabolism of flesh, was enormous (6,383 ml or 14 lb); the amount lost concomitantly from the catabolism of fat was negligible (367 ml or 0.8 lb). The catabolism of 1,664 g of protein which resulted in a loss of 18.3 pound of body weight, yielded only 6,828 cal. If the fasting subject had been in protein and carbohydrate equilibrium the 6,828 calories would have been derived from adipose tissue with a loss of only 1.82 pound (0.83 kg). The categoric conclusion can be drawn that the caloric equivalent of one pound of body weight lost when an individual is fasting, is markedly less than when he is on a low-calorie diet, but in protein and carbohydrate equilibrium.

Clinical observations confirm the error in Joslin's thesis. If a person who requires 2,500 cal/day to maintain caloric equilibrium is on a daily diet containing 1,000 cal, he will give a daily deficit of 1,500 cal or a weekly deficit of 10,500 cal. According to Joslin, such a person should lose 7 pounds of body weight per week. From practical experience we know that this does not occur. In Strang's[3] patients the loss of 0.6 pound of body weight/day resulted from a deficit of 2,100 cal. If Joslin's figure of 1,500 cal for the caloric equivalent of one pound of body weight lost were accepted, they should have lost 1.4 pounds instead of 0.6 pound actually lost.

Utilizing indirect methods for the estimation of body composition Keys *et al.*[6] in a study of individuals who had not previously fasted, found that on a high calorie diet the caloric equivalent of 1 kg of the tissue mass gained was 6,180 cal. They also found that the character of the tissue mass gained in rehabilitation by previously starved men was quite different from that gained by well-fed men who simply ate to excess. Whereas, about two-thirds of the weight gain of the latter was pure fat, in the starved men the gain in early rehabilitation was made up of only 10 to 20 per cent fat.

DISCUSSION

Certain definite conclusions may be made from the observations and studies described above. The average obese individual who is placed on a low-calorie diet for the purpose of weight reduction, is practically in protein and carbohydrate (glycogen) equilibrium. The caloric deficit in these patients is made up almost entirely by the catabolism of fat. The theoretical value of one pound (454 g) of adipose tissue is 3,750 cal.[1] In clinical studies by Strang *et al.*[5] on weight reduction on obese individuals who were on a low-calorie diet a value of 3,500 cal was obtained for each pound of body weight lost. The conclusion can be drawn that 3,500 is the caloric value of one pound of body weight lost.

It must be stressed that it is fundamental that the protein content of the low-calorie diets employed in weight reduction be kept high. If they are so low as to permit appreciable negative nitrogen balances, there will be considerable loss of water. This will result in a decrease in the caloric equivalent of 1 lb of body weight lost and consequently a more rapid rate of loss in weight. This, is, however, a spurious loss because it has no permanency. After the desired weight loss has occurred and

the patient has been placed on a diet adequate to maintain caloric equilibrium, the protein stores which have been depleted during the period of negative nitrogen balance will be replenished. For every pound of protein replenished there will be an increase of four pounds in body weight. Thus, even though the patient is in caloric equilibrium, he will continue to gain weight until the protein stores have been completely restored.

The value of one pound of body weight gained is also 3,500 cal if there is no deposition of protein or carbohydrate (glycogen). After a prolonged fast, replenishment of the depleted protein and carbohydrate stores will be associated with a concomitant marked deposition of water which will decidedly reduce the caloric equivalent of one pound of body weight gained. When the individual is again in protein and carbohydrate equilibrium, all foods in excess of the amount necessary to maintain caloric equilibrium will be converted into fat and the caloric equivalent of one pound of body weight gained will be about 3,500.

In prolonged fasting there is a marked negative protein balance with a concomitant loss of large amounts of water. There is also a negative carbohydrate (glycogen) balance with a loss of water of the same order. However, since glycogen stores are small, this factor is not important. The enormous losses of water, combined to a small degree with the fact that the caloric values of protein and carbohydrate are less than half the value of a similar amount of fat, resulted in a large reduction of the caloric value of one pound of body weight lost. Thus Joslin[6] calculated that each pound of body weight lost by Benedict's subject, who fasted for 31 days and lost 30 pounds had a value of 1,500 cal, which is less than half of that determined for one pound of body weight lost in clinical weight reduction.

The following two points are relevant to this subject. A person who weighs 300 pounds and is putting out 3,000 cal a day in bodily activities may be placed on a reducing diet. Because of hunger he may reduce his activities to, perhaps, 2,000 cal/day. Thus he would lose weight more slowly than he would on a regimen of activity equal to that before the diet.

As weight loss occurs, caloric expenditure decreases. For example, a male, age 40 years, 300 pounds in weight, 70 in. in height, has a daily basal caloric expenditure of 2,265 cal. If the weight were reduced to 200 pounds, the basal caloric expenditure would drop to 1,995 cal, a difference of 270 cal/day or 8,100 cal/-month. Thus, on the same regimen of activity and diet, he would lose two and one-third pounds more per month when the body weight is 300 pounds than when it has been reduced to 200 pounds.

It is of importance to discuss the weight curve of patients undergoing weight reduction on low-calorie diets. Many observers have emphasized the fact that although a patient may burn many grams of stored fat per day he does not necessarily lose weight every day. The curve of the daily weight change is a series of ups and downs with frequent periods of no apparent weight change. As has been repeatedly demonstrated, these irregularities are intimately associated with the storage and release of relatively large quantities of water. The influence of this phenomenon on the true weight loss is minimized if the period of observation is sufficiently long. It is possible to predict with gratifying precision the weight loss to be expected from the deficiency in exogenous calories.

The fact that 1 g of flesh has a value of approximately 1 cal and 1 g of adipose tissue a value of approximately 8 cal is of considerable practical importance. Thus, in certain conditions where parenteral alimentation has to be resorted to for any considerable length of time, it is important to supply protein derivatives so as to keep the negative nitrogen balance as low as possible, and prevent large weight losses from the loss of flesh. This is of even greater importance in chronic diseases such as anorexia nervosa, carcinoma of the esophagus, carcinoma of the stomach, and other conditions where alimentation is very difficult. Here the primary consideration is to furnish a diet high in protein. If a large negative nitrogen balance is permitted, the loss of weight from the catabolism of flesh will be large with a yield of only a small number of calories.

SUMMARY AND CONCLUSIONS

The low-calorie diets on which individuals are placed for the purpose of weight reduction should be high in protein so that protein and glycogen will be approximately in equilibrium. The calorie deficit will be made up chiefly by the catabolism of fat. Under the circumstances (high-protein intake) the caloric equivalent of one pound of body weight lost is approximately 3,500 cal.

The caloric equivalent of one pound of body weight gained is dependent on the state of the protein and carbohydrate (glycogen) stores. If the protein and glycogen stores have been depleted as the result of fasting, their replenishment will be associated with a concomitant deposition of large quantities of water. During this period the caloric equivalent of one pound of body weight gained will be markedly less than 3,500.

When a state of protein and glycogen equilibrium is reached, all food ingested in excess of the amount necessary to maintain caloric equilibrium will be converted into fat (adipose tissue). The caloric equivalent of one pound of body weight gained will then be 3,500.

In fasting there are always decided negative protein and carbohydrate (glycogen) balances. The catabolism of protein and glycogen is associated with losses of large quantities of water. The caloric equivalent of one pound of body weight lost in fasting (negative nitrogen balance) is always markedly less than 3,500.

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Letter to the Editor

Sorbitol and Vitamin B_{12} Absorption

Dear Sir:

In a recent issue of The American Journal of Clinical Nutrition Schilling[1] commented editorially on the need for data showing the effects of D-sorbitol on vitamin B_{12} absorption in patients unable to produce intrinsic factor. Such data have been obtained in patients with pernicious anemia at this institution, and recently reported.[2] D-sorbitol did not enhance vitamin B_{12} absorption in these patients, either when given with 2 μg of vitamin B_{12} as previously reported,[3] or with 30 μg of the vitamin.

It was speculated that perhaps D-sorbitol worked by enhancing the action of intrinsic factor, and so the same patients were given a D-sorbitol-hog intrinsic factor concentrate-vitamin B_{12} combination (using both 2 and 30 μg amounts of the vitamin) The addition of D-sorbitol had absolutely no further enhancing action on vitamin B_{12} absorption over the enhancement observed with hog intrinsic factor concentrate alone. Furthermore, D-sorbitol did not enhance vitamin B_{12} uptake by everted sacs of rat small intestine,[4] using an incubation system[5] in which hog intrinsic factor concentrate produces marked enhancement of vitamin B_{12} uptake.[6] These findings offer strong indirect support for Schilling's suggestion that perhaps D-sorbitol stimulates gastric secretion of intrinsic factor. They also provide a cogent argument against the use of D-sorbitol "to enhance vitamin B_{12} absorption" in any patient who lacks intrinsic factor or in whom intrinsic factor is ineffective (i.e., sprue, etc.) at least until further studies completely clarify the role of this agent.

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Diet Therapy

Adapting Therapeutic Diets to the Eating Patterns of Italian-Americans

MARJORIE CANTONI, M.P.H.*

FOR Italian-Americans, as for Italians everywhere, eating is an important part of living. Planning a diet for any member of a group with such a high interest in food calls for some understanding of his cultural background.

As is true with other ethnic groups, Italian-Americans have largely Americanized their eating habits. Such factors as difficulty in obtaining traditional food products, the pressure of American merchandizing, the custom of "eating out," and even the school-lunch programs have all contributed, in some measure, to this change. Consequently, younger Italian-Americans eat favorite Italian dishes only on special occasions. The main concern of this article is with Italian-Americans over 40 years of age who still retain the Italian cultural eating pattern and who are at an age when they are more likely to need therapeutic diets.

In most instances, this paper will refer to Italians as a homogeneous group, pointing out differences and variations from non-Italians. However, certain markedly different characteristics can be found within this ethnic group.

During its early history, Italy was an aggregate of independent States, which engendered an individualism in each State. This individualism still prevails. Dialect differs from one area to another; there are different patron saints for each locale and favorite dishes are quite individual. There also appears to be a constant undercurrent of rivalry among provinces and regions, each one having a favorite tradition or custom to boast about, to be proud of, and to defend. Because of their historic background, the people from a single village, town, or province feel a close relationship perpetuated through many generations—it is felt even by those who have come to the United States. When Italians discover that they or their ancestors are *paesani*, (i.e., from the same *paese* or region) there is an indescribably close bond, next only to blood relationship.

In large part, Italians emigrated to the United States seeking the opportunity to work and earn a modest living. Most of them came from farms and fishing villages where the land and sea did not yield enough for the dense population. They settled in American urban areas tending, until quite recently, to congregate in certain sections of large cities. Many found work in industry and others settled on the Pacific Coast in areas where the fruit growing seasons and climate are similar to those of Italy.

Italians plan their meals like many Europeans: a light breakfast, substantial mid-day meal, and a lighter meal in the evening. For families whose members stay away all day, mid-day and evening meals are often reversed. In Italy, the middle and upper classes take a

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siesta after the noon meal and those who work or are shopkeepers have a longer work day to compensate for the two to four hour siesta at noon. It is natural that eating patterns and the custom of leisurely meals were retained by Italians coming to this country.

Knowing this background, it is evident that a successful approach to dietary prescription begins by evaluating the Italian way of eating and then suggesting such changes as are necessary for adequate nutrition. This will be more successful than trying to impose an arbitrary pattern of a substantial breakfast, light lunch, and complete early evening dinner. The patient's clinical condition, of course, dictates the foods that must be restricted or encouraged.

TYPICAL FOODS OF THE ITALIAN-AMERICAN DIET

Bread is the mainstay of every Italian meal. The shape of the loaf is characteristic of the different Italian provinces, but all are white, crusty loaves and very substantial. Bread to the Italian is more than just a foodstuff; it signifies a way of life.

There are Italian expressions which reflect this high regard for bread. For example, it is most complimentary to be called in the Italian idiom "as good as bread." Another Italian expression is *compatico*. Roughly translated, this word means "that which goes with bread," implying that most foods accompany bread which is considered the principal food.

Most Italians in the United States buy bread from local Italian bakeries. High-protein flour, water, yeast, and salt are the ingredients used. Only where states have adopted a bread enrichment law that governs all breads will the Italian bread contain additional iron, thiamine, riboflavin, and niacin. Whether or not bread is enriched will greatly influence the quantity of these vitamins and iron in the diets of Italian-Americans who eat Italian bread regularly.

Pasta is the all-inclusive term for the various forms and shapes of macaroni, spaghetti, and egg noodles. They constitute a basic group of foods for the Italian people. Closely associated are such rice dishes as *risotto alla Milanese* which is rice cooked in broth and flavored with saffron, parmesan cheese, onion, and mushroom; *polenta*, a thick yellow corn meal mush served plain, with meat and vegetables or made into a casserole dish containing pieces of sausage, tomato sauce, and grated cheese. The rice, corn meal, and egg noodles are common in the Northern Italian's diet, while spaghetti is the characteristic pasta in the South.

Pastasciutta can be any pasta served with gravy or sauce and the name distinguishes it from pasta served in broth or soup.

It is not uncommon to find Italian-American women making their own egg noodles. These are cut into various shapes—small squares, long narrow strips, wider strips, then dried for cooking and eating during the week.

MEAL PATTERNS

Broth with noodles or pastasciutta is the first course of the principal meal. If broth is served, chicken or meat, vegetables, and salad follow as the second course.

The meats and poultry that appear quite frequently on the Italian-American dinner table are: roast chicken, pieces of chicken baked with oil, garlic, salt, and pepper; chicken cacciatora lightly browned in olive oil then simmered in wine and tomato sauce; veal as cutlets, scallopine, or with tomato sauce, Italian meat balls, meat loaf, roasted or fried Italian pork sausage, in addition to plain roast meat, baked or fried chops, and Italian style stews.

Some of the favorite vegetables are broccoli, escarole, spinach, string beans, zucchini and other squash, asparagus, egg-plant, artichokes, peppers, and canned tomato used in sauces. A favorite way of preparing many of the green vegetables is to cook in water, drain, then add either olive oil or olive oil and vinegar.

On the average, families who eat in this fashion, eat smaller quantities of meat than many Americans and extend meat with vegetables, sauces, bread crumbs, and by serving a variety of foods.

Insalata, a salad of escarole, chicory, lettuce, dandelions, endive, or romaine, is eaten almost daily, sometimes twice daily. These greens may be served alone or mixed with tomatoes, onions, green peppers, and celery. Invariably, the dressing is olive oil and vinegar, salt, pepper, and garlic. Wine vinegar is preferred by many families. If a large quantity of oil is

used, a blend of a less expensive oil, such as cottonseed, and olive oil is frequently found in the household cupboard.

Even though Italians are quite satisfied with fresh fruit for dessert, in this country, they tend to have more pie and cake than is customary in Italy. Rich Italian pastries are served on Sundays and special occasions.

Minestrone, a substantial soup made with vegetables, ceci (chick peas), and pasta or *pastafasioli* (bean soup), is served as the main dish for the lighter meal. Few other items appear on the table after soup, except perhaps, cheese or Italian cold cuts and salad. Italians, even in the second and third generations, prefer the expensive Italian-style cheeses. *Parmesan* and *romano* are hard varieties for grating, and *ricotta* and *mozzarella* are soft cheeses used in cooking or eaten with bread. Cold cuts such as *salami*, *mortadella* (a bologna-type sausage), *coppa* (a highly peppered sausage), and *prosciutto* (Italian-cured ham) are also preferred to the American varieties in spite of their higher cost.

Seasonings

Characteristic Italian flavors in food are developed by a variety of vegetables, herbs, wine, cheese, oil, salt pork, and tomato puree. Green peppers, onions, and celery are often blended together in sauces. Garlic, red pepper, parsley, oregano, rosemary, basil, nutmeg, and saffron are used singly or several may be combined in the same dish. For example, spaghetti sauce will have in it garlic, sometimes parsley, oregano and/or nutmeg. Southern Italian cooking features red pepper, making the sauce highly spiced and hot. The herb saffron gives the characteristic yellow color and delicate flavor in *risotto alla Milanese*. Nutmeg is added to meat balls, pepper steak, and sometimes to veal cutlets.

One method of cooking which differs from the American custom is the slow simmering of meats and sauces on top of the stove. Oil, salt pork, or butter is heated in a heavy cooking pan, finely cut celery, onion, parsley, garlic, green pepper, or any combination of these are sautéed, then the meat or fish is browned lightly; tomato puree, wine, or other liquid is added to finish the cooking on low heat. Basically all the different kinds of spaghetti sauce, whether ground beef, clams, or vegetables, are made this way. Italian meat balls, chicken, cubes of round steak, veal stew, and chops are often simmered as described. The amount of oil or fat varies with the cook as does the combination of vegetables and seasonings.

Foods for Holiday Occasions and for Fast Days

A *festa*, i.e., any holiday, or a wedding or christening, for instance, is celebrated with a variety of foods and dishes not a part of everyday eating. All types of pastry, *torta* (cake), and cookies are made at home or purchased from the Italian bakery. The menu is much more elaborate and may begin with an unusual pasta such as *ravioli*, *tortellini*, *cannelloni*, baked *lasagne*, or *manicotti* (variously formed noodle dough filled with mixtures of meat, vegetables, or cheese). This is usually followed by roast chicken, veal cutlets, roast lamb, or pork. A variety of vegetables, such as broccoli, artichokes, mushrooms, cauliflower, asparagus, eggplant—each cooked in a favorite Italian style—and a mixed green salad round out the meal. A dry red or white wine is often served.

The religious fast days, such as Friday, Lent, and especially fast days preceding Christmas and Easter, are occasions for spaghetti with a fish sauce made of anchovy, clam, or tuna. A meatless tomato sauce is another favorite. Dishes featuring eggs, cheese, vegetables, or dried beans are often substituted on these meatless days.

Some Italian families prepare eels or squid in delectable dishes, but generally they buy the many kinds of locally caught fish. For canned fish their selection is limited mainly to tuna, sardines, and anchovies. One unusual fish dish is *baccala:* salted, dried codfish which has been soaked in water several days and prepared carefully to the taste of the family. One way is to sauté it in olive oil, then simmer with tomato sauce and seasonings. Because of the long preparation involved, *baccala* is featured in the United States only on rare or special fast days.

Italians believe that what they eat is nutritious and healthful. They view American de-

hydrated and canned foods with some suspicion and misgivings. When sick, they prefer to have simple foods, such as chicken broth to which egg and cheese have been added to enhance its nutritional value. Wine is thought to have therapeutic value for the debilitated person. A special dessert sometimes given to a person in delicate health is *zabaglione*—soft custard made of egg yolks, white wine, and sugar.

Italians place a high value on food preparation and flavor in cooking, in contrast to many Americans who may think first of vitamin content. However, the basic Italian diet need not lack essential nutrients. The physician who takes a few minutes to ask his Italian patient about his eating habits will be rewarded with interesting answers. Such questions as: "What do you usually eat?"; "How do you plan your meals?"; "What did you eat all day yesterday?" will lay a firm foundation for instructing the patient and helping him to follow a diet.

TYPICAL MEAL PATTERNS

Although there are cultural differences throughout all sections of Italy, differences are most marked between the northern and southern parts. A first-generation Italian-American woman from the northern part of Italy gave the following 24-hour food history:

Breakfast

Orange juice
Prunes
Coffee with milk and sugar

Noon

Chicken broth with noodles and grated cheese
Italian sausage roasted in the oven
Insalata—green salad seasoned with olive oil, vinegar, salt, pepper, and garlic
Italian bread (no butter)
Fresh pear
Coffee with half milk

Evening

Frittata—omelette made with eggs, cheese, bread crumbs, asparagus, and seasonings
Broccoli sautéed in olive oil and garlic
Insalata
Italian bread
Plain cake

A man from the southern part of Italy now living in the United States ate these foods for one day:

Breakfast

1 whole orange
1 cup of milk and coffee
2 oz Italian bread

Mid-morning

1 cup of coffee

Noon meal

2 large servings of spaghetti with marinara sauce (olive oil, garlic, tomatoes, basil, salt, and pepper), and grated romano cheese
6 oz fried sea bass
1 peach and 1 banana
1 glass wine
Coffee

Mid-afternoon

Coffee

Evening meal

2 oz prosciutto (Italian cured ham)
Insalata (escarole, oil, and vinegar)
4 oz Italian bread
1 glass wine

Nutritive Adequacy of the Typical Diet

Many of the earlier nutrition references on the Italian eating habits in the United States pointed out the lack of milk and limited meat in the diet and inferred that the diets did not measure up to the N.R.C. Recommended Dietary Allowances. However, in the analysis of nutrients in the two cases, of the northern Italian woman's and the southern Italian man's actual 24-hour food history, there was no apparent shortage of essential nutrients.

Following is a brief résumé:

Nutrients	N.R.C. Recommended Allowance—woman over 65 years	Northern Italian woman over 65 (Actual 24-hour but typical food history)	N.R.C. Recommended Allowance—man 45 years	Southern Italian man—45 years (Actual 24-hour but typical food history)
Calories	1,800.	1,985.	2,900.	2,520.
Protein (g)	55.	81.	65.	109.
Calcium (g)	0.8	1.	0.8	0.86
Iron (mg)	12.	11.	12.	12.
Vitamin A (i.u.)	5,000.	6,890.	5,000.	12,546.
Thiamine (mg)	1.	1.	1.5	2.1
Riboflavin (mg)	1.4	1.5	1.6	1.7
Niacin (mg)	10.	22.	15.	21.
Vitamin C (mg)	70.	155.	75.	123.

It can be generalized that Italian-Americans who eat a variety of food, though following an Italian eating pattern, are getting nutrients which meet the N.R.C. Recommended Dietary Allowances, revised 1953.

ATTITUDE TOWARD ILLNESS

Patients of Italian background tend to regard their physicians with great respect and confidence. Italians generally are uninhibited in their expression of pain and will groan, moan, look perfectly miserable, and implore the physician to give them medicine to relieve pain. As soon as they feel better, they are likely to resume their habitual happy outlook with little concern about the actual cause of their illness.

Considering this attitude toward pain, it is quite understandable that, in general, as long as Italians have painful or uncomfortable symptoms, they will follow diet prescriptions; but as they recover, they may not be as cooperative and may find it difficult to understand the rationale for adhering to the diet. If as many of the familiar Italian foods as possible, particularly bread, are included in the prescribed diet, greater cooperation can be expected. Careful explanation of the therapeutic diet will help the patient understand why some foods must be restricted; it may also help him appreciate the beneficial, long-term effects of following the diet.

Many Italian men are quite familiar with the preparation of numerous dishes, and can often give a detailed account of what they eat, the quantities, and even recipes. This is to be expected in a culture where so much enjoyment is derived from eating. If the therapeutic diet is interpreted by planning meals similar to those of the whole family, with only the necessary modifications, the patient is likely to be not only appreciative but motivated to follow the diet more closely.

Bland Diet

The bland diet, such as that prescribed for gastrointestinal disorders, can be liberalized, simplified, and individualized to the needs of each patient. In most cases, the Italian patient can continue to have his Italian white bread, chicken broth with noodles or rice, eggs prepared in easily digestible forms, ricotta, and many of the pasta dishes mixed with mildly seasoned sauces. Oregano, basil, rosemary, and other similar herbs will add zest to the foods without irritating the digestive tract.

Plain milk is difficult for most Italian-American patients to accept. A little coffee, chocolate, or vanilla flavoring, whichever is allowed, may be the adjustment necessary for patient cooperation.

The variety of raw vegetables and fruits may be replaced by cooked or canned ones containing a minimum of fiber, seeds, or skin. Fried foods and foods high in fats, such as *frittata* (made of egg, grated cheese, and bread crumbs), fried meats, sliced cold cuts, can be replaced by dishes which are baked, broiled, or simmered on top of the stove. The fats and oils must be kept to a minimum for easier digestion. Such spices as red pepper which may cause distress will be restricted. Now that the rationale of a bland diet has changed, foods are no longer arbitrarily forbidden, but are allowed as they can be tolerated by each patient.

Sodium-Restricted Diet

Sodium restricted diets need not be an extreme hardship to the Italian-American patient. Herb cookery is so much a part of his cuisine that he perhaps uses less salt in cooking and at the table than many other Americans. He can be encouraged to continue using fresh garlic, oregano, basil, saffron, and the many herbs with which he is familiar.

Italians prepare many dishes from basic ingredients, rather than using canned and frozen food or prepared mixes, which simplifies adherence to a sodium-restricted diet. For example, salads are dressed by pouring oil and vinegar from separate containers; no salt or sodium products need be considered as would be the case in commercially prepared French dressing or mayonnaise.

Some of the basic Italian foods can be allowed in a sodium-restricted diet with only a few recipe adjustments. While tomato sauce is being made, for instance, a portion can be removed before the salt is added and saved for the member of the family on a sodium-restricted

diet. Spaghetti, macaroni, noodles and, of course, rice can be eaten if no salt is added in cooking or at the table. The flavor of liver and meats is enhanced with lemon juice as well as with herbs and sodium-free seasonings. Nutmeg is often used in meatballs or tomato sauce. Italians also use lemon juice or vinegar to flavor many vegetables.

During the holiday seasons, sweet (unsalted) butter is one of the ingredients for torta and special desserts. Sweet butter is considered a delicacy, and consequently will be acceptable to the average Italian patient in this country.

The greatest hardship to the patient when his diet must be severely restricted in sodium will be the curtailment of Italian bread. One solution is to suggest that Italian bread without salt be baked at home; otherwise the patient must resort to buying American low-sodium bread.

Another difficulty is in limiting Italian cheese which is often used in cooking and enhances the flavor of foods. However, ricotta or mozzarella without salt are available in some cities with large Italian populations. Omission of desserts and pastries will not be too troublesome for the Italian-American patient if he can have fresh fruits.

It is advisable to ask the Italian patient what laxatives and home remedies he uses for stomach upsets, indigestion, and flatulence. "Brioschi," a lemon flavored antacid, is basically sodium bicarbonate. Another home remedy used by many Italians is "Fernet" an alcohol and herb mixture. The label suggests its use as a "stimulant to appetite and a mild laxative," but since no sodium compounds are named in the long list of ingredients, it need not be restricted.

Fat-Restricted Diet

Recent dietary studies suggest that population groups whose diets are high in fat have a higher incidence of coronary heart disease. Other research projects are concerned with the study of more definite fat characteristics and their effect on serum cholesterol levels in man. Up to the present time it has not been determined conclusively whether the characteristic in question is that the fat originates from vegetable or animal, or that it contains essential fatty acids, or that the fat is saturated or unsaturated. However, a physician may prescribe a low-fat diet or want to modify the kinds of fat in a patient's diet for the treatment of various clinical conditions.

As is usual in low-fat diets, fried foods and fatty meats need to be restricted but lean meat, fish, plain vegetables, and simple desserts may be specified. The Italian patient can continue to eat his Italian bread, since it is generally made with an insignificant amount of fat or none at all. Vegetables and fruits can play a prominent part in his diet if the vegetables are served with only vinegar or with oil taken from the day's allowance. Vegetable soup and broth with pasta can be eaten if the patient is instructed to remove all the fat from soup first. Sauce for spaghetti can be made using lean meat with only the amount of fat allowed in the diet.

Fruit desserts as well as light desserts using the amount of eggs and milk allowed in the diet may be encouraged. The rich pastries, high in cream, butter and other fats, and those that must be fried ought to be eliminated. "Sweet bowknots," *farfalleti dolci*, are made from an egg yolk, flour, and sugar and fried in lard or hydrogenated fats. *Cannoli* are pipe-shaped pastries, fried and filled with sweetened ricotta. Obviously these special Italian pastries should be stricken from a low-fat diet list.

Diabetic Diet

Many physicians are using the "Meal Planning and Exchange List" for interpreting the diabetic diet to patients. The meal planning booklet can be helpful to Italian-American diabetic patients, now that the text takes into account foods used by groups with different cultural backgrounds. The Italian patient may select the types of fats, vegetables, meats, breads, or bread exchanges that he prefers or to which he is accustomed.

Accurate measurement of different foods needs to be emphasized until the diabetic patient knows them thoroughly. For example, Italian bread can be estimated by weighing one ounce and visualizing it as an equivalent to one slice of American bread.

While the Italian patient is learning his diet, he should be encouraged to ask questions and discuss any foods which may be questionable. Thus, items in his daily bill-of-fare can be planned in terms of the prescribed diet. The Italian-American may decide to eliminate minestrone unless he knows that the vegetables on his diet list can be used in soup. If chick peas, lentils, or macaroni are added to minestrone, they can be exchanged for bread, since the bread exchange list notes that $^1/_2$ cup of lentils, dried beans, or macaroni has about the same caloric value as one slice of bread.

Other adjustments can be made in a similar way using the "exchange list." If meat is allowed on the diet, Italian tomato sauce may be satisfactory if made with the permitted amount of oil or fat. Baked lasagne requires greater scrutiny but may be eaten by the diabetic patient applying these exchanges: cooked lasagne or noodles in place of bread, cheese and meat for meat allowed, and oil for fat.

The older Italian patient may find it difficult to drink milk unless it is flavored with coffee, used in custard or a similar dish. In adapting the exchange list, one ounce of cheese and one bread exchange can be suggested in place of 8 ounces of milk.

Diets for Weight Control

The Italian patient may take the problem of obesity rather lightly. Food and meal times are both enjoyable and satisfying. Withholding food is seldom used as a disciplinary measure with children.

Because of these attitudes toward food, prescribing a realistic reducing diet for the Italian-American patient is difficult. To achieve any degree of success, he must be strongly motivated to lose weight and he must be willing to adjust his diet accordingly. At the same time, provision must be made for him to continue some of the deep-seated satisfactions and enjoyment derived from food.

Limiting the quantities of fat and fried foods, and advising smaller portions of pasta dishes and bread, rather than changing the diet completely, will fulfill these requirements. Lean meats, fish, eggs, cheese, vegetables, salads, and fresh fruits should play an important part in his reducing diet. The discussion of diabetic diets and fat restriction is pertinent for reducing diets and those recommendations can be incorporated.

Similarly, most other therapeutic diets can be adapted by the physician for his Italian-American patients within their general pattern, which has for these many centuries, accorded food a place of high importance in both their tradition and daily living.

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Diet Lists

FROM TIME TO TIME we will publish diet lists which seem to have clinical usefulness. Minor variations exist in all standard diets; nevertheless these are representative and may be considered useful guides. Publication does not necessarily imply complete agreement with all details.

The following diet list may be obtained from the U. S. Vitamin Corporation, 250 East 43rd Street, New York, N. Y.

Low Cholesterol, Low Fat, High Protein Diet

Two important points should be remembered in following this diet: (1) You should avoid animal fats as found in fat meats, lard, cream, whole milk, and egg yolk. (2) Generous portions of the allowed protein foods should be eaten three or four times a day. Small quantities of vegetable fats may be used in cooking and at the table, but fried foods should be avoided. Certain foods are forbidden because of their high cholesterol content even though they are not fat.

Food	*Not allowed*		*Allowed*	
Meats and other protein foods	Fried meats	Mackerel	Lean meats (trim off fat)	
	Fat meats	Herring	Veal	Chicken
	Fresh pork	Salmon	Beef	Turkey
	Egg yolk	Shad	Ham	Lamb
	Fish canned in oil		Non-oily fish	
	*Liver	Brains	Haddock	Flounder
	*Sweetbreads	Tuna	Cod	Blue fish
	*Shell fish	*Bacon	Halibut	Bass
	Sardines	*Kidney	Fresh water fish	
	Butterfish		White of egg only	
Fats	Butter		Vegetable shortenings and margarine (used sparingly)	
	Lard			
	Suet and other animal fats		Crisco, Spry	Peanut oil
			Olive oil	Soybean oil
			Corn oil	
Cereals	Noodles made with eggs		Any cereals (preferably whole grain)	
	Breads and hot breads made with eggs		Bread, wheat cakes made without eggs	
			Macaroni, spaghetti, noodles made without eggs	
Dairy products	Whole milk, cream		Skim milk	
	Most cheeses		Buttermilk	
	Ice cream		Cheese made from skim milk (dry cottage cheese)	
Fruits	None		Any fruit or juice	
			Avocado and nuts (in small amounts)	
Vegetables	None		Potatoes, baked without butter, or mashed with water or skim milk	
			Any vegetable prepared without oil or fat	
Desserts and sweets	Rich desserts made with fat, cream, egg yolks		Gelatin desserts	
			Ices and sherbets	
	Rich candy made with butter		Tapioca and rice puddings made with fruit and juices	
	Ice cream			
	Pastry		Plain cookies	
	Chocolate in all forms		Angel food and other cakes made without egg yolks and butter	
			Jams, jellies, honey	
			Candy made without butter or cream	
Beverages	Whole milk		Tea, coffee, cereal drinks	
	Alcohol		Buttermilk, skim milk	
			Soft drinks	

Soups, etc.	Cream soups	Clear broth
	Gravies	Vegetable soup made without whole milk or fat
	Cream sauce	
Miscellaneous	Olives	Salt, spices
	Mayonnaise	Vinegar
	Oily dressing	Popcorn without butter
	Fried foods	Relishes, pickles
	Potato chips	Catsup
	Brewers yeast	Low fat yeast extracts

Sample Menu

Breakfast	*Lunch*	*Dinner*
Grapefruit	Split pea soup	Tomato juice
2 poached egg whites	Crackers	Lean ham
2 slices whole wheat bread	Lean beef—roast	Broccoli with lemon
Jelly	Green vegetable	Lettuce salad with dry cottage cheese
Whole grain cereal	Rice cooked in clear broth	Whole wheat roll
1/2 cup skim milk	Tomato salad	Sherbet made with egg white
Coffee (without cream) or weak tea	Gelatin dessert	Coffee (without cream) or weak tea
	1 glass buttermilk	

If practical, a glass of skimmed milk should be taken midway between breakfast and lunch, and again between lunch and dinner. Carrot sticks and fresh fruit may be taken to allay hunger between meals.

Vegetable margarine should be substituted for butter and used sparingly, 1 pat a meal. Coffee and tea are allowed with sugar, but not with cream.

* NOTE: The following foods are of high nutritive value, but are omitted from the diet because of their moderate cholesterol content. Any one (but not more than one) of these foods may be eaten once a day in amount not to exceed that suggested:

Liver—3 oz
Kidney—3 oz
Sweetbreads—3 oz
Whole milk—1 glass (6 oz)
Lobster– 3 oz
Canadian bacon—3 oz
Peanut butter—1 tbsp

Reviews of Recent Books

Practical Clinical Chemistry: A Guide for Technicians, ed. 2, by Alma Hiller. Thomas, Springfield, Ill., 1957, pp. 265, $6.50.

This book contains the routine methods employed in the chemistry laboratory of the Presbyterian Hospital of Chicago. Beginning with three introductory chapters (General Laboratory Procedures, Standard Solutions of Acid and Alkali, and Photometry), the volume contains detailed descriptions of acceptable methods for amylase, direct and total bilirubin, bromsulfalein, calcium, carbon dioxide combining capacity, cephalin flocculation, chloride, total cholesterol, creatinine, icterus index, total nitrogen, nonprotein nitrogen, inorganic phosphorus, alkaline and acid phosphatase, protein, sugar, sulfonamides, and uric acid. Following the adequate index is a section of removable outlines of each method, which may serve as a handy reference for the laboratory technologist.

Differences from the first edition include (1) minor changes in the bromsulfalein, icterus index and nonprotein nitrogen methods, (2) instead of the Bloor method for total cholesterol, substitution of the Pearson technic utilizing *p*-toluenesulfonic acid, (3) modification of the Folin-Wu sugar method to include the more stable arsenomolybdate color reagent of Nelson, (4) substitution of the shorter Caraway method for uric acid for that of Kern and Stransky, and (5) the addition of the Moore caramelization technic for rapid estimation of urinary sugar.

Dr. Hiller chose the nonprotein nitrogen method rather than urea nitrogen because of simplicity in technic; this reviewer would have preferred the latter. The chloride method is the excellent one of Van Slyke and Hiller.

The fact that a second edition has been issued speaks for the deserved popularity of a book by a well-known, capable worker in the field of clinical chemistry.

H. F. WEISBERG

Calcium Metabolism, by J. T. Irving. Wiley, New York, 1957, pp. 177, $2.75.

This little book covers a wide range of subjects pertaining to calcium, which the author enthusiastically proclaims in the opening sentence as the most important inorganic element in the body. The extent of coverage of the world literature is exemplary. Possibly the most important contribution of this book is the almost complete marshalling of the references pertaining to calcium metabolism. Only one notable area is excepted in this regard, and surprisingly enough it concerns the "Extra-Skeletal Functions of Calcium." This engaging area of calcium metabolism, the last chapter before the summary, is covered in less than two pages without a single reference. This omission is unwarranted even though the author points out that 99 per cent of body calcium is in the bones and teeth and 1 per cent in other cellular and bodily activities.

Two of the most valuable chapters are concerned with bone formation and bone chemistry and physics. In these chapters the author has been particularly concerned with a clear, terse presentation of highly complex subjects and has succeeded admirably with a more critically appraising discernment than is evidenced in some other parts of the book. In other chapters the reader has the feeling that various references have been included because they touched upon the subject, not because they contributed to our knowledge of the subject. On these occasions, an aura of superficiality often pervades several paragraphs and leaves the reader with the desire that a higher level of criticism had been attained and that more pertinent details had been included from the meritorious studies with the deletion of non-contributory investigations.

Even with the foregoing weaknesses, which are not sufficiently serious to detract from the main theme, this book will prove useful to all concerned with various aspects of calcium metabolism because it is the best current compilation of information on this subject.

J. H. SHAW

Heart Disease—Cause, Prevention, and Recovery by Philip S. Chen. The Chemical Elements, South Lancaster, Mass., 1958, pp. 189, $3.00.

This small and well printed volume is allegedly written for the layman who wishes to have a thorough understanding of the nature of heart disease.

Part 1, written jointly with Philip S. Chen, Jr., serves as an introduction to structure and function of the heart and briefly defines the main types of heart disease. It is clearly written and substantially correct except for misleading values for cardiac output.

Part 2, entitled "Cause and Contributory Factors of Heart Disease," includes an introduction to the biochemistry of fats, but consists mainly of a rather uncritical collection and dogmatic presentation of facts, opinions, and advice regarding the possible role of

food, diet and living habits in health and disease. The author's personal conviction that meat, milk, animal fats, smoking, and lanolin are major health hazards is everywhere apparent and no effort is spared to cite supporting opinions.

Part 3 discusses the prevention of heart disease and is designed to extoll the virtues of the soy bean in the place of meat, milk, and eggs. There follows a chapter citing the bible's recommendations of a vegetarian diet and discussing in detail the Seventh Day Adventist's teaching with regard to diet.

This volume in essence presents the case for the nutritional cause and prevention of atherosclerosis and disease of the coronary arteries. Although many sources are quoted, these appear to have been selected on the basis of conclusions rather than merit of the evidence. Furthermore, although some references are given in full, most references are incomplete. In view of the contents of the book, the title may be considered misleading inasmuch as it does not reflect the main topic discussed. Because of evident bias and dogmatism, it is difficult to recommend this volume to any lay or professional group. However, much material of interest has been brought together in a manner useful to the discerning student of the relation of nutrition to health and disease.

W. H. ABELMANN

Low Fat Cookery, by Evelyn S. Stead and Gloria K. Warren with an introduction by Eugene A. Stead, Jr., M.D. and James V. Warren, M.D. McGraw-Hill, New York, 1956, pp. 184, $3.95.

This book was written to provide easy-to-prepare recipes for persons desiring to restrict dietary fat and for those finding it necessary to reduce fat for weight reduction, hypertensive vascular disease, coronary artery disease, atherosclerosis, or diabetes. Mrs. Stead has had much meal planning practice in her role of homemaker, while Mrs. Warren is an experienced therapeutic dietitian.

This book contains 165 recipes along with their respective fat contents, including some for appetizers or hors d'oeuvres, soups, meats, fish and poultry, cheeses and cheese spreads, salads and salad dressings, sandwiches, sauces, vegetables, desserts, beverages, and breakfast items. An herb chart, tables of fat content of foods, and a week's sample menus on a 25-g and 50-g fat level are added aids. Other highlights are a good index, clever illustrations, and good organization.

On the whole, the recipes are set up in a manner that is easy to follow. In a few, more logical order of ingredient listing would add to the clarity of the whole recipe.

Most of the ingredients are readily available and within the average budget. The reviewer feels, however, that name brand products are listed too often, and with no indication as to whether a satisfactory result could be obtained with another brand. This may render some recipes useless because of unavailability of ingredients or possible cooking failures when inadequate substitutions are made.

The table of fat content appears accurate, although listing values to tenths of grams when foods are measured and not weighed may be unnecessary. Real help may be gained from the herb chart.

The menus illustrate that a low-fat diet can be attractive and nutritionally adequate, but no information on how to plan meals is included. Since using fat content as the sole criterion for menu planning could be disastrous, some material on the subject would be valuable.

This writer feels that the book would be of limited use to the diabetic, since the recipes are not translated into exchanges or any other method of diabetic diet calculation. Restriction of dietary fat *per se* is still of debatable value for a person with cardiac disease. This book will have its primary usefulness for the homemaker who wishes recipe supplementation for the instructions given by the physician or dietitian.

EMMA SEIFRIT

Hormones in Blood, ed. by G. E. W. Wolstenholme and E. C. P. Millar (Ciba Foundation Colloquia on Endocrinology, Volume XI), Little, Brown, Boston, 1957, pp. 416, $9.00.

This book serves to disseminate information concerning hormones in blood to endocrinologists and scientists who were not so fortunate as to attend this meeting. Outstanding investigators present data regarding chemical and biologic analyses of various hormones in blood, factors which influence their concentrations, and mechanisms of their transport. The chapters devoted to studies of adrenocortical hormones are of considerable interest and provide much information about synthesis, release, binding to plasma proteins, and degradation of these steroids. The difficulties inherent in present methods of determining content of neurohypophyseal hormones in blood are well presented. Discussions of thyroid hormones in blood as well as plasma insulin concentration are enlightening.

Endocrinologists, biochemists, and physiologists will find this book of valuable assistance when considering problems related to analysis of hormones in body fluids. It will also serve as a modern reference on certain aspects of the biochemistry and physiology of many hormones.

ALBERT B. EISENSTEIN

International Congress of Gastroenterology. *Fifth Meeting, London, 1956,* ed. by Harold Edwards. S. Karger, Basel, 1957, pp. 762, S.Fr. 78.

The papers presented at the International Congress of Gastroenterology in London in 1956 and previously published in *Gastroenterologia,* Vol. 86, Nos. 3, 4, and 5 (1956) are more permanently preserved in this single volume. The published articles fall into several subjects: nonmalignant conditions of the esophagus; premalignant conditions of the alimentary tract; a miscellaneous group; and ulcerative colitis.

Among the papers on esophageal disorders, the American reader is reassured to find reports on generally familiar material from C. S. Code, Mayo Clinic, F. J. Ingelfinger and P. Kramer, Boston, and E. C. Texter and C. J. Barborka, Chicago. Less familiar is the work of J. Nauta, of Holland, on the closing mechanism between the esophagus and the stomach which describes an unusually extensive investigation combining anatomic and physiologic studies and includes photographs of the cardiac opening of the dog taken from the stomach side. The impressive number of rather basic investigations reported from European centers indicates that the recently renewed interest in the esophagus has not been confined to this country.

The papers on premalignant conditions of the alimentary tract are, with the exception of a report on exfoliative cytology by Dr. J. B. Kirsner and associates, of the University of Chicago, entirely from foreign sources. The provocative observations of R. Doll, of Great Britain, on environmental factors in the etiology of cancer of the stomach is the outstanding contribution.

The group of short miscellaneous papers constitutes a condensed summary of much of the important investigations in gastroenterology during the past few years. Included are papers by G. B. Jerzy Glass and his associates, of New York, on paper-electrophoretic analysis of gastric juice, by Sheila Sherlock and her associates, of Great Britain, on the treatment of hepatic coma, by J. Waldenstrom, of Sweden, on the diagnosis and pathology of carcinoidosis, by W. Sheldon, of Great Britain, on celiac disease, by F. Hollander, of New York, on enzyme inhibitors in the therapy of gastroduodenal ulcer, and by J. N. Hunt, of Great Britain, on the influence of low concentrations of acid in test meals on gastric secretion.

The reports on ulcerative colitis include summaries by a number of well-known figures such as H. L. Bockus and associates, of Philadelphia, J. A. Bargen, of the Mayo Clinic, and C. W. Wirts, of Philadelphia. G. Lumb, of Great Britain, presents a compact summary of his experience with rectal biopsy in ulcerative colitis. There are a number of papers on the natural history of the disease.

The official languages of the Congress are English, French, German, and Spanish, however, English predominates. The papers are illustrated with good reproductions of photographs and charts so that it constitutes an excellent summary of the world literature in the field of gastroenterology during the past several years.

J. B. HAMMOND

Books received for review by THE AMERICAN JOURNAL OF CLINICAL NUTRITION are acknowledged in this column. As far as practicable those of special interest are selected, as space permits, for a more extensive review.

Nutrition for Practical Nurses, ed. 2 by Phyllis S. Howe, Saunders, Philadelphia, 1958, pp. 219, $2.75.

The Year Book of Endocrinology (1957–1958 Year Book Series), edited by Gilbert S. Gordon, Year Book Publishers, Chicago, 1958, pp. 381, $7.50.

Transactions of the Sixth Meeting of the International Society of Geographical Physiology. S. Karger, Basel, 1958, pp. 642, S.fr. 67.60.

The Cerebrospinal Fluid. Production, Circulation and Absorption (Ciba Foundation Symposium), edited by G. E. W. Wolstenholme and C. M. O'Connor, Little, Brown, Boston, 1958, pp. 335, $9.00.

Nutrition and Diet Therapy for Practical Nurses by Lillian Mowry, Mosby, St. Louis, 1958, pp. 165, $2.50.

Nutrition for You by Robert S. Goodhart, Dutton, New York, 1958, pp. 215, $4.50.

Diseases of the Esophagus by J. Terracol and Richard H. Sweet, Saunders, Philadelphia, 1958, pp. 682, $20.00.

Processed Plant Protein Foodstuffs, edited by Aaron M. Altschul, Academic Press, New York, 1958, pp. 955, $26.00.

Hormone Production in Encodrine Tumors (Ciba Foundation Colloquia on Endocrinology, Vol. XII), edited by G. E. W. Wolstenholme and Maeve O'Connor, Little, Brown, Boston, 1958, pp. 351, $9.00.

Is Overweight Curable? by Leo B. Janis, Tioli Publishing Co., Toledo, 1958, pp. 164, $4.75.

Diseases of the Liver and Biliary System, ed. 2 by Sheila Sherlock, Thomas, Springfield, Ill., 1958, pp. 719, $11.50.

Abstracts of Current Literature

SPECIAL ASPECTS OF DIETS

The caloric intake recommended by the National Research Council seems to be considerably more than that consumed by young women in an evaluation of self-selected diets. The ability of the subjects of the following report to maintain their weights on a low-dietary-energy equivalence suggests that the recommended intakes are higher than necessary. Similar conclusions have been derived from studies dealing with protein allowances.

The Energy Value of Self-Selected Diets Consumed by Young College Women. A. N. Davis and F. I. Scoular. *J. Nutrition* 61: 289, 1957.

As a routine procedure in the elementary nutrition course at North Texas State College, individual dietary records are kept and their nutritive value estimated. The low caloric intake of these college women was evident from the first. Since the caloric value of self-selected diets is of importance in assessing the adequacy of other nutrients, it seemed wise to determine quantitatively the caloric value of college women's diets. Consequently, the determination of the caloric value of the foods consumed was made a part of the present long-time metabolism study of young college women consuming self-selected diets.

The average height, 163.9 cm, and the average weight, 56.7 kg, of 89 Texas college women are greater than those given by the National Research Council for 16- to 20-year-old girls and for 25-year-old women.

Sixty-six of the women were within the weight range for height, 11 were underweight and 12 overweight. The average daily caloric intake was 2,103 ± 211 and 2,101 ± 183 for group I (under 20 years) and 2,158 ± 216 and 2,141 ± 206 for group II (over 20 years) when distributed on the basis of the height and the weight of the individuals. Thirty-three of group I and 22 of group II (60 per cent of 89) consuming 13 cal/cm maintained their weights on such an intake.

B. SURE

The Protein Metabolism of Young College Women Consuming Self-Selected Diets. F. I. Scoular, A. N. Davis, J. K. Pace, A. B. Rankin, and G. J. Boshart. *J. Nutrition* 61: 297, 1957.

The average daily protein intake of 171 young college women on self-selected diets ranged from 19 to 113 g protein with an average of 52 g/day. The subjects absorbed from 65 to 98 per cent of the ingested protein with an average absorption value of 85 per cent. Six of the young women consumed 75 or more grams of protein daily, 66 consumed from 55 to 75 g and the remainder (102) less than 55 g/day. Sixty-six per cent (112) of the women were in positive protein balance on these intakes.

Group I (under 20 years) ingested an average of 0.80 ± 0.18 g protein/kg while group II (over 20 years) consumed 0.88 ± 0.19 g/kg of protein. When the ingested protein was distributed according to the height of the subjects, the average protein intake was 0.33 ± 0.08 g/cm for group I and 0.34 ± 0.06 g/cm for group II.

B. SURE

It is of interest to note the close correspondence between the caloric intakes of the women in the following report and those of the female students in the abstracts of this series.

The Energy Expenditure and Food Intake of Middle-aged Glasgow Housewives and their Adult Daughters. J. V. G. A. Durnin, E. C. Blake, and J. M. Brockway. *Brit. J. Nutrition* 11: 85, 1957.

During recent years there has been a very considerable increase in the available information on energy

expenditure in relation to activity. Measurements have been made on individuals of differing ways of life in several countries. Yet few studies have attempted to cover the whole 24-hour period, as opposed to estimates covering the working time only. The estimates of calorie requirements and the decrement for age, where women are concerned, have been largely based on guesswork and on the relatively few dietary studies reported.

The individual intakes and expenditures of energy of middle-aged, middle-class housewives and of their adult working daughters were measured daily for 7 consecutive days. Twelve mother-daughter pairs were studied. The girls all worked as saleswomen in a large department store in Glasgow. The mean daily intake of calories was 2,100 cal for the mothers and 2,225 for the daughters; the mean expenditures were 2,090 and 2,255 cal/day, respectively. A table is given to show the individual daily variation in the intake and expenditure of calories. The pattern of the activities of both groups is also shown. Possible explanations for the quite moderate demands of housework on energy expenditure are discussed.

Since the activities of mothers and daughters were not unlike in terms of energy expenditure, it was possible to derive the decrement in calorie expenditure due to age: a decrement of 3 per cent for each decade over the age of 25 years is in keeping with the results of these investigators. B. SURE

The Diets of Middle-Aged Glasgow Housewives and their Adult Daughters. J. V. G. A. Durnin, E. C. Blake, and J. M. Brockway. *Brit. J. Nutrition* 11: 94, 1957.

The food intake of a group of 12 middle-aged, middle-class Glasgow housewives and that of their 12 adult daughters was measured individually throughout seven consecutive days. Details of the various constituents of the diets are given and the adequacy of the diets is discussed. Most of the essential nutrients were taken in what is believed to be sufficient quantity, but several mothers and daughters had low intakes of calcium, iron, riboflavin and ascorbic acid. B. SURE

The control of food intake by activation or inhibition of hypothalamic centers has been the subject of much recent investigation. Among the important factors influencing the appetite are changes in heat production and the arteriovenous glucose differences.

The Specific Dynamic Action of Food and the Satiety Mechanism. R. Passmore and F. J. Ritchie. *Brit. J. Nutrition* 11: 79, 1957.

Food intake is controlled by two centers in the hypothalamus, a "feeding centre" which promotes feeding and "a satiety centre" which inhibits it. Several workers have contributed to the anatomic discovery of these centers. It has been postulated that "animals eat to keep warm and stop eating to prevent hyperthermia." However, it is generally agreed that changes in heat production may be only one of several stimuli to which the centers react.

Energy exchange in man during the first hour after a meal has been investigated. A rise in rectal temperature and a probable rise in average skin temperature have been demonstrated, but these were so small as to make quantitative assessment very difficult. Oxygen consumption increased immediately after a meal and the specific dynamic action of food appears to have no latent period. The results are consistent with Brobeck's hypothesis (*Yale J. Biol. & Med.* 20: 545, 1948) that increased heat production is an important part of the satiety mechanism. Other factors besides specific dynamic action must also contribute to the satiety mechanism and individual variations are important. B. SURE

The statement is frequently made that improved nutritional intake is an important factor in reducing fetal mortality and preventing certain complications of pregnancy. However, in the absence of gross deficiencies in protein, calories or accessory food factors in the diets of the pregnant patient, there is little evidence that healthy child-bearing is affected.

Technique and Perspective in Clinical and Dietary Studies of Human Pregnancy. A. M. Thomson. *Proc. Brit. Nutrition Soc.* 16: 45, 1957.

None of the common disabilities of pregnancy are deficiency diseases of classic type. Some of them seem to be expressions of inefficient physiologic adaptation to pregnancy, due in some measure to chronic and probably nonspecific inadequacy or imbalance of diet. The correlations between food intake during pregnancy and the outcome of pregnancy are so elusive that they are far from easy to demonstrate even by *ad hoc* survey methods. This is true at least for populations where the average level of nutrition is reasonably high, such as those of Britain and America. Nevertheless, improvement in the diet of pregnant women was responsible for the dramatic reduction of fetal mortality in Britain during the recent war, and there is no reason to think that further improvement is impossible or that the effort would be unrewarding. Again, there is ample confirmation of the commonsense supposition that well-fed girls have a much better chance than those who are ill-fed of becoming well-grown and healthy mothers and of having an uncomplicated pregnancy and labor. A very high standard of nutrition among infants, children, and probably also adolescents is therefore essential to healthy childbearing. It cannot be achieved without educating and helping mothers on the importance of good diets for themselves. B. SURE

CURRENT DEVELOPMENTS IN DIABETES

A vast literature on the sulfonylurea compounds has accumulated in a brief period. It seems likely that these drugs have a primary action in releasing insulin from surviving beta cells of the diabetic pancreas; a secondary effect of delaying the release of glucose from the hepatic cells has been described. The latter action may depend upon the liberation of endogenous insulin. Clinical results are discussed herein. The selection of suitable patients for drug treatment has not been made successfully on the basis of a brief test indicated in the first paper.

Recent Studies Relative to the Treatment of Diabetes: Special Reference to New Oral Antidiabetic Drugs. R. H. Williams and E. D. Henley. *A.M.A. Arch. Int. Med.* 99: 501, 1957.

The authors present a good description of the sulfonyl-urea compounds. In their experience, about 50 per cent of diabetic patients show a relatively good response to these drugs. However, less than 10 per cent of the patients with juvenile diabetes respond while the majority of those over age sixty seem to respond well. The smaller the required insulin dosage before drug therapy, the better the results. Selection of the appropriate patients is greatly assisted by subjecting the patient to a four-hour test of a hypoglycemic response to tolbutamide. The authors conclude that present investigations will likely lead to the provision of new oral drugs which are equally or more active, less toxic, and have greater clinical advantages than the present sulfonylureas. S. O. WAIFE

Clinical Studies of the Hypoglycaemic Action of the Sulphonylureas. W. J. H. Butterfield, J. L. Camp, C. Hardwick, and H. E. Holling. *Lancet* 1: 753, 1957.

The effect of seven days' treatment with sulfonylurea (with and without insulin) on various carbohydrate tolerance tests, arterio-venous differences in blood sugar levels, blood α-keto-acid levels, peripheral blood flow, and the hyperglycemic response to injected glucagon was studied in 17 female and 12 male diabetic patients of various types.

In 22 patients the fasting blood sugar fell more than 15 per cent. The only statistically significant effect of treatment on the tests was in the intravenous glucose tolerance test, where the blood sugar level fell below the fasting level at 60 and 90 minutes. There was a small but significant fall in the level of α-keto acids. An increase in peripheral blood flow associated with the treatment complicated the interpretation of the arterio-venous differences. The treatment did not significantly affect the response to an intravenous dose of 1 mg of glucagon.

It is argued that the sulfonylureas do not affect the absorption of glucose or the phosphorylation and dephosphorylation in either intestinal wall or liver. Nor do they apparently act by affecting the response to insulin or to the blocking of other hormones. Their mode of action remains obscure. F. E. HYTTEN

Several investigators have described an increase in plasma and urinary corticoids in diabetic acidosis and coma. The increase in steroid levels develops after the onset of metabolic decompensation. In the absence of acidosis, steroid levels have often been described as normal; however, in the following report elevation of the corticoids was found in non-acidotic patients.

The Role of the Pituitary-Adrenal-System in the Physiopathology of Diabetes. A. Goth, M. Goth, L. Lengyel, C. Savely, and E. Stadler. II. (Relation of the Plasma 17-Hydroxycorticosteroid Level to Blood Sugar Fluctuations in Diabetes Mellitus). *Ztschr. Vitamin- Hormon- y. Fermentforsch.* 8: 230, 1956.

In non-compensated diabetes accompanied by large blood sugar fluctuations, urinary corticoid excretion is 146 per cent and the plasma corticoid level 123 per cent in excess of normal—even in cases where there is no acidosis. In cases where the blood sugar fluctuations did not exceed 100 mg/100 ml (i.e., well controlled cases) the plasma corticoid level and urinary corticoid excretion were found to be normal. In severe, non-compensated diabetes, the plasma corticoid level increases from morning to evening as a result of the daily blood sugar fluctuations, while in normal subjects it decreases. AUTHORS

Increased glucose-6-phosphatase may be an adaptive enzymatic response to insulin deficiency since insulin administration will reduce its activity.

Glucose-6-Phosphatase Activity in Human Diabetes. S. J. Patrick and J. A. Tulloch. *Lancet* 1: 811, 1957.

The activity of glucose-6-phosphatase which catalyses the conversion of glucose-6-phosphate to free glucose in the liver is affected by a number of metabolic states. Little previous investigation has been attempted in humans with diabetes.

Thirteen diabetic patients of various types, and seven non-diabetic persons were submitted to liver biopsy and the enzyme activity measured on the biopsy material. There was increased activity of glucose-6-phosphatase in the diabetic livers, compared with the non-diabetic. Stabilization of the diabetic state resulted in a decreased activity. The results are not discussed.
F. E. HYTTEN

The conversion of fructose and galactose to glucose by appropriate enzymatic pathways within the liver results in glycosuria in the presence of insulin deficiency.

Utilization of Fructose, Galactose and Glucose by Diabetic Rabbits. S. Banerjee and E. R. Divakaran. *Am. J. Physiol.* 188: 543, 1957.

Fructose, galactose, and glucose were fed to normal

and alloxan-diabetic rabbits. Some of the animals received an intravenous injection of dihydroergotamine prior to the feeding of fructose. The levels of these sugars and true glucose were estimated in samples of blood collected for varying periods after the administration of these sugars. These sugars were also determined in the urine of the animals. Fructose and galactose were removed from the blood very rapidly in both the normal and diabetic rabbits and insignificant amounts of these sugars appeared in the urine. There was a considerable increase in the true blood glucose level and increased amount of glucose appeared in the urine of diabetic rabbits after fructose or galactose was fed. Dihydroergotamine partly suppressed this hyperglycemia after the administration of fructose. Alloxan-diabetic rabbits, in spite of their normal ability to initiate the metabolism of fructose and galactose, either transform a considerable amount of these sugars into glucose or spare the utilization of blood glucose. Superiority of these sugars as alternate source of energy is questioned. AUTHORS

Pregnancy Complicated by Diabetes Mellitus: a Review of 119 Cases. A. E. M. Stevenson. *Brit. M. J.* 2: 1514, 1956.

The results of 15 years' experience in Belfast with pregnant diabetic patients are reported in detail. The results are claimed to be unsatisfactory. Total fetal loss was 30 per cent (25 per cent excluding abortions). Hormone therapy was of no value. "There is at present no known method which will prevent intrauterine death and allow pregnancy to go to term."

Recommendations are made which it is suggested would substantially reduce fetal loss. These are, broadly, accurate and skilled control of the diabetes from early in pregnancy, preferably in the hospital, and premature delivery by caesarean section.

It is surprising that the excellent results of Brandstrup, *et al.* (previous abstract in 1956), published in Denmark where many of the suggestions here have been in use for some years, are not mentioned.

F. E. HYTTEN

Aspirin and Diabetes Mellitus. J. Reid, A. I. MacDougall, and M. M. Andrews. *Brit. M. J.* 2: 1071, 1957.

Rheumatic fever and diabetes mellitus are said to occur together very rarely, but this unusual combination appeared in a young male diabetic at a Glasgow hospital. On salicylate treatment in hospital his urine was sugar-free and his fasting blood sugar was normal although he had stopped taking his usual insulin. Diabetic signs reappeared after his discharge from hospital.

The possibility that this phenomenon was due to the salicylate was investigated and clearly established in this and seven other diabetic patients. Four were of the mild obese type and three of the lean more severe type. When salicylate was given in doses of 1 to 1.6 g at four-hour intervals the fasting blood sugar, glycosuria, glucose-tolerance curve, and ketonuria became normal. Symptoms also disappeared although there were some side effects from the salicylates, principally tinnitus and deafness. The serum salicylate was high; it averaged about 40 to 45 mg/100 ml during treatment. The basal metabolic rate was considerably raised by the salicylate which is known to be a peripheral-acting metabolic stimulant, and the evidence presented in this inquiry suggests that the site of action of the aspirin in diabetes is the tissues.

There is a promising new line of research offered by this experience since aspirin seems to be at least as effective and less toxic than the sulfonylureas.

(*Ed. Note:* Ingle and others reported on salicylates in experimental diabetes a number of years ago.)

F. E. HYTTEN

CHOLESTEROL ABSORPTION AND METABOLISM

One of the methods proposed for the lowering of serum cholesterol levels is that of inhibiting the enteric formation of the esterified cholesterol. The process of esterification is required for absorption of most of the endogenous or exogenous cholesterol.

Factors Facilitating Cholesterol Absorption from the Intestine via Lymphatic Pathways. G. V. Vahouny, I. Fawal, and C. R. Treadwell. *Am. J. Physiol.* 188: 342, 1957.

The lipid fractions of thoracic duct lymph in unanesthetized rats were determined following intragastric administration of saline-albumin emulsions containing various combinations of cholesterol, taurocholate, and oleic acid. Sodium taurocholate or oleic acid alone produced significant increases in the total lipid, neutral fat and phospholipid fractions, but had no effect on the level of free and ester cholesterol. Administration of cholesterol alone was without effect on any of the fractions. The combination of taurocholate and oleic acid gave the same levels of lipid fractions as when they were administered singly except that there was an elevation of ester cholesterol indicating increased absorption of endogenous cholesterol. Cholesterol plus taurocholate or oleic acid produced the same increases in the fractions as the salt or acid alone except that with both combinations there were highly significant increases in the total and ester cholesterol fractions. Administration of the three factors together gave further increases in all fractions except neutral fat and free cholesterol. The amount of free cholesterol was constant throughout all groups, even in those in which there was absorption of exogenous cholesterol. The percentage of ester cholesterol in the total cholesterol of lymph ranged from 66 to 81 with the higher percentages in the groups where cholesterol absorption occurred. The esterification of the "extra" cholesterol in lymph due to cholesterol absorption ranged from 86 to 92 per cent. It is suggested

that essentially all of the cholesterol transferred from the intestinal lumen to the lacteals is esterified.

AUTHORS

Role of Pancreatic Digestion in Cholesterol Absorption. T. M. Lin, E. Karvinen, and A. C. Ivy. *Am. J. Physiol.* 190: 214, 1957.

The exclusion of pancreatic juice had no significant effect on elimination of endogenous cholesterol in the rat but increased it slightly in three dogs. Forty per cent of the dietary cholesterol was absorbed without and with pancreatic exclusion in the presence of a fat-free diet. Hence, pancreatic juice is not specifically necessary for the absorption of cholesterol. Pancreatic exclusion had no effect on the absorption of either dietary cholesterol or fatty acid, or both, when oleic and palmitic acid were fed. This indicates that any effect pancreatic exclusion may exert on cholesterol absorption when a fat containing diet is fed depends on the change in the utilization of the fat resulting from the exclusion. In the case of corn oil, triolein, trielaidin and tallow but not with tripalmitin, pancreatic exclusion was followed by an increased fecal elimination of both fatty acid and cholesterol. The increment of fatty acid elimination was large enough to dissolve the excess cholesterol excreted in the rats with pancreatic exclusion, except in the case of trielaidin. The only statistically significant decrease in the absorption of dietary cholesterol which resulted from pancreatic exclusion occurred when one of the unsaturated fatty acid esters, namely, corn oil, triolein, or trielaidin was the fat fed. These observations fail to show that pancreatic cholesterol esterase plays a specifically essential role in the absorption of free dietary cholesterol. AUTHORS

Vegetable sterols and other related factors may inhibit cholesterol absorption by interfering with ester formation necessary for absorption of the latter compound. However, the effects of long-term administration of the interfering substances may prove detrimental due to their absorption and deposition in tissues.

Inhibitory Effect of "Isocholesterol" on the Absorption of Cholesterol. M. M. Best and C. H. Duncan. *Circulation Res.* 5: 401, 1957.

"Isocholesterol," a mixture of 30-carbon sterols found in wool fat possesses a free hydroxyl group on carbon-3, a characteristic common to the known steroid inhibitors of cholesterol absorption. Isocholesterol differs from other inhibitors in that it does not form insoluble precipitates with digitonin.

The addition of 5 per cent isocholesterol to a low cholesterol diet for 13 days had no significant effect on liver or serum cholesterol level in the rat. The addition of 5 per cent isocholesterol to a 1 per cent cholesterol diet exerted a marked inhibitory effect on the accumulation of cholesterol in the liver and was followed by a fall in serum cholesterol.

The findings are presented as evidence for interference with intestinal absorption of cholesterol by one or more of the constituents of isocholesterol.

W. H. ABELMANN

Use of Δ 4-Cholestenone to Reduce the Level of Serum Cholesterol in Man. G. M. Tomkins, C. W. Nichols, Jr., D. D. Chapman, S. Hotta, and I. L. Chaikoff. *Science* 125: 936, 1957.

Preliminary studies are presented on the effects of prolonged administration of cholestenone (4-cholestene 3-one) on plasma lipids. One gram of the substance fed to a dog every eight hours for 17 days led to a fall in plasma cholesterol value of 100 mg/100 ml to 70 on the eighth day and 55 mg on the seventeenth day. In another dog, the level of plasma cholesterol fell from 115 mg/100 ml before feeding to 70 mg/100 ml. Similar reductions in the level of plasma cholesterol were observed in chickens that were fed chow with added 1 per cent cholestenone. In connection with the prolonged feeding of the substance in birds, it was found that the level of total sterols, in contrast to cholesterol level, is not reduced in the plasma and large amounts of cholestanol accumulate in plasma and other tissues. Furthermore, the prolonged feeding of this substance has been shown to induce arteriosclerosis in rabbits and chickens. The authors call attention to the dangers that may result from prolonged administration of large amounts of the steroid-like cholestenone, which is converted in the animal body to an "arteriosclerosis-inducing" sterol (dihydrocholesterol). In experiments of this type it is also important to know the level of total steroids in plasma, as well as cholesterol.

S. O. WAIFE

Reduced amounts of lymph cholesterol in animals fed albumin with a lipid emulsion suggests that the esterification of the sterol is inhibited by the protein or by peptides formed by proteolysis within the intestine.

Changes in Lipid Composition of Lymph During Cholesterol Absorption in the Rat. G. V. Vahouny and C. R. Treadwell. *Am. J. Physiol.* 191: 179, 1957.

Time studies of the appearance of lipid fractions in the thoracic duct lymph of rats were performed following the administration of emulsions containing cholesterol, oleic acid and sodium taurocholate. The influence of added protein on lipid levels and the total nitrogen of lymph was also studied. Addition of albumin to administered saline was without effect on lymph flow, lipid fractions or total nitrogen. In those animals receiving cholesterol, oleic acid, and taurocholate, in addition to albumin, a rapid increase in total lipid was evident during the initial three hours, followed by a gradual fall in this fraction to near preabsorptive levels at the end of nine hours. Comparable changes were observed in the ester cholesterol, neutral fat, and phospholipid fractions. Animals receiving a similar emulsion lacking albumin displayed less marked increases in the lipid fractions which were, however, prolonged

throughout the experimental period. In contrast to the other lipid fractions in this group, the amount of lymph cholesterol for the 24-hour period was significantly greater than in the comparable group receiving albumin. During cholesterol absorption in both experimental groups, the increase in total lymph cholesterol was attributable almost entirely to an increase in the esterified fraction, which comprised between 84 to 92 per cent of the absorbed sterol. AUTHORS

Weekly Variations in Serum Cholesterol Levels of Monkeys. D. Kritchevsky and R. F. J. McCandless. *Proc. Soc. Exper. Biol. & Med.* 95: 152, 1957.

In male Cynomolgus monkeys weighing 4 to 6 lb serum cholesterol levels were determined in duplicate by the method of Tinder for two weeks before sit. sterol feeding, during six weeks of sit. sterol feeding and for six weeks after discontinuation of feeding. One gram of sitosterol was given just before the animals' single daily feeding. Before this feeding the serum cholesterol levels ranged from 86 to 141 mg/100 ml; during the treatment with sitosterol levels of from 84 to 186 mg/100 ml were noted, and after discontinuation 92 to 257 mg/100 ml. The cholesterol values of the treated animals failed to correlate with the cholesterol level of the same individual animal before treatment.

These observations seem to be important. The findings show that more data covering prolonged periods of time must be accumulated before an evaluation of the over-all cholesterol variations can be made, and thus before definite conclusions can be drawn on the cholesterol-depressing effect of sitosterol. M. SILBERBERG

Lard vs. a Vegetable Fat in Relation to Liver and Serum Cholesterol. R. Okey and M. M. Stone. *J. Am. Dietet. A.* 32: 807, 1956.

When cholesterol stores in liver and serum were assessed in matched pairs of rats fed lard or vegetable fat as 13.5 or 15 per cent of the diet for 28 to 56 days, these authors found that "lard-fed males tended to store somewhat more liver cholesterol than did those fed equal percentages of vegetable fat. Differences were lessened by an increase in the protein content of the diet. Lard-fed females showed smaller differences in liver cholesterol than did males. Serum cholesterols were, however, consistently higher in females."

This "pilot" study attempted to compare the effect of animal and vegetable fats both with and without added cholesterol on stores of cholesterol in rats' livers and in the serum. Data from two series of studies are reported. In Series I, 13.5 per cent fat and 15 or 30 per cent protein were fed and in Series II, 15 per cent fat and 15 per cent protein were used. Male rats weighing 150 g and females of 135 g were fed 28 to 30 days in the first series whereas weanling rats were fed for seven weeks in the second study. When cholesterol was fed, 1 g replaced 1 g sucrose in the diet. Data for males and females are treated separately and for Series I mean values for liver lipids and cholesterol are reported. For Series II serum cholesterol values are reported as well. In Study I, differences in liver lipid and cholesterol were not very great in sub-groups fed lard or vegetable fat. Males fed lard plus cholesterol had definitely higher liver cholesterol levels than did those fed vegetable fat (327 ± 32 mg/liver vs. 248 ± 40). For male rats fed 30 per cent protein corresponding values were 106 ± 22 and 92 ± 21. Females fed 30 per cent protein had higher liver cholesterols with vegetable fat than with lard (95 ± 7 for lard-fed and 105 ± 45 for vegetable fat-fed).

Animals fed 15 per cent fat from weaning showed more evidence of cholesterol storage on the lard diet (85 ± 16 mg/liver vs. 45 ± 6 for males and 23 ± 3 vs. 20 ± 1 for females). Mean serum cholesterol values were always higher for females than for males (77 mg/100 g for males and 86 for females). Cholesterol-fed females on cottonseed oil had higher serum cholesterols than those fed lard with cholesterol (109 ± 7.6 mg 1/100 g vs. 131 ± 11.4).

The reviewer found the comparisons difficult to follow and believes the findings would have been more significant if food intakes and weight gains for animals had been reported. Data were reported for one group with as few as four animals in contrast with some groups containing ten. No statistical analysis of the data was reported so that it is difficult to judge the significance of reported differences. Some of the variations observed might have been reduced by use of the paired-feeding technic since there is demonstration that levels of fat and protein and intake of preformed cholesterol all affect serum and liver cholesterol levels.

J. M. SMITH

Hypercholesterolemia induced by the feeding of thiouracil and fat supplements is associated with the occurrence of experimental atherosclerosis in dogs. In the absence of thyroid suppression, cholesterol deposition is not observed suggesting that the vascular lesion may be produced by a primary alteration of the mucopolysaccharides in the myxedematous state.

Response of Dogs to Long-Term Cholesterol Feeding. K. H. Shull and G. V. Mann. *Am. J. Physiol.* 188: 81, 1957.

Hypercholesteremias averaging 495 and 570 mg/100 ml were maintained in two female dogs for periods of three and one-half and four years, respectively, by cholesterol feeding. The serum beta-lipoprotein levels were also grossly elevated throughout these periods. Neither the level of fat calories nor the calorie balance appeared to affect significantly these serum lipid levels. Gross and microscopic examinations performed at autopsy on these two dogs failed to reveal any abnormalities in the cardiovascular system or liver. AUTHORS

Utilization of Calories From Alcohol and Wines and Their Effects on Cholesterol Metabolism. A. F. Mor-

gan, L. Brinner, C. B. Plaa, and M. M. Stone. *Am. J. Physiol.* 189:290, 1957.

The utilization of calories for growth by young rats given an adequate diet, free access to drinking water and supplements of 15 or 20 per cent alcohol solutions or wines of the same alcohol concentration was equal to that of rats receiving no alcohol if the alcohol calories were calculated as 75 per cent physiologically available. When no additional water was given along with the alcohol and wine solutions or when water intake was restricted to the amount taken by the alcohol groups, the intake of diet and growth were at once decreased about equally in the water restricted, wine and alcohol groups. When 1 per cent cholesterol was added to the diet all the rats grew better than on the basal diet and water restriction had less unfavorable effects. The liver fat of the alcohol groups was higher than that of the others on both basal and cholesterol diets. Both liver and adrenal cholesterol were much increased by the exogenous cholesterol in all groups but least in the wine fed animals. Hamsters under similar conditions were little affected by water restriction, but growth was decreased on the cholesterol diet and grossly fatty livers developed containing about half the lipid content as cholesterol. Serum cholesterol was much increased, least in the wine-fed groups. Restriction of water intake by rats given alcohol solutions appears to account for most of the effects of chronic alcohol ingestion heretofore reported. AUTHORS

Failure of Alpha-Phenylbutyrate and Beta-Phenylvalerate in Treatment of Hypercholesterolemia. D. S. Frederickson and D. Steinberg. *Circulation* 15:391, 1957.

The authors previously had reported that sodium α-phenylbutyrate (phenylethylacetic acid, sodium salt) or sodium β-phenylvalerate inhibit the rate of conversion of acetate-1-C^{14} to cholesterol and fatty acids *in vitro* and *in vivo*, but do not affect the serum level of cholesterol in rats.

Ten hypercholesterolemic and one normocholesterolemic patients were given sodium α-phenylbutyrate (nine courses) and/or sodium β-n-phenylvalerate (five courses) in doses of 17 to 120 mg per kg for periods of 21 to 84 days. Only one patient showed a significant fall in serum cholesterol, while at least four showed an equally significant rise. Two additional patients developed a toxic dermatitis necessitating withdrawal of sodium phenylvalerate.

In explanation of the failure of these compounds to lower serum cholesterol, the authors suggest that inhibition of acetate activation may not affect synthesis of cholesterol from glucose and fatty acids via acetylcoenzyme A without degradation to the level of free acetate. W. H. ABELMANN

Elevation of serum lipids is observed in various clinical states characterized by hypercholesterolemia, such as nephrosis, myxedema and diabetes. Inhibition of lipolytic lipase or excessive mobilization of serum lipids may elevate the serum levels of fats providing a transport medium for cholesterol which remains in the lipemic serum largely as a β-lipoprotein.

Mechanism Underlying Hypercholesteremia Induced by Triton WR-1339. M. Friedman and S. O. Byers. *Am. J. Physiol.* 190:439, 1957.

Sequential changes in the plasma lipids of rats injected with Triton were studied. It was observed that following a dose of Triton, the accumulation of triglyceride (neutral fat), cholesterol, and phospholipid proceeded in plasma for about 36 hours, after which time a rapid diminution began. Excess triglyceride began both to accumulate and to disappear, respectively, before the other two substances. During the phase of accumulation, the excess cholesterol in plasma was not found to be reflected by any increase in cholesterol either in the liver tissue or in hepatic lymph. This lack of diffusibility of plasma cholesterol disappeared during the period of recovery. Injection of Triton retarded the escape of both injected triglyceride and phosphatide from the plasma of the liverless rat. Hypercholesteremia could be induced in the liverless rat by Triton injection if triglyceride, phosphatide or both were given with the Triton. It is suggested that the hypercholesteremia occurring after injection of Triton is due to the latter's ability to retain excess triglyceride and phospholipid which then in turn mobilizes and sequesters cholesterol from both extrahepatic and hepatic sources. AUTHORS

New procedures and technics for clinical use and investigative study of the problems involved in disorders linked with lipid and cholesterol derangements are sorely needed.

Alpha- and Beta-Lipoprotein Cholesterol, Method of Rapid Quantitative Determination. F. S. Nury and E. R. B. Smith. *Clinical Chemist* 3:110, 1957.

The method described is an improvement of a method developed by Largan, Durrum, and Jencks. The older method required little serum, but took more than a day to perform, and special equipment was necessary. After hanging-strip paper electrophoresis, the strips are dried. A "marker" strip is stained with Sudan Black, rinsed and dried. The lipid pattern of each stained marker strip is divided into sequents which best separate α and β bands, including lipoprotein. Unstained strips of identical segments are prepared. The unstained segments are extracted in test tubes with chloroform-methanol; the solvent is evaporated off and glacial acetic acid is added to the residue in each tube. The tubes are cooled, and a ferric chloride-sulphuric acid reagent is added. Incubation at 56° C is necessary for 17 to 23 min. After cooling and centrifugation to remove air bubbles, the optical densities are read in a Coleman Jr. spectrophotometer at 560 mμ, with the reagent blank set at zero optical density. Optical density data can be corrected for eluate retained in the extracted

paper. Calculations are based on the value of a cholesterol standard, after corrections for the elution blank.

If electrophoretic separation of the serum into alpha- and beta-lipoprotein fractions is omitted, this method can be used to estimate total serum cholesterol in less than two hours. Comparison of this total serum cholesterol method with that of Sperry and Webb in 40 sera gave a mean insignificantly lower than the older standard method. Statistical analysis indicated good agreement between methods. E. COHEN

A Stable Iron Reagent for Determination of Cholesterol. H. L. Rosenthal, M. L. Pfluke, and S. Buscaglia. *J. Lab. & Clin. Med.* 50: 318, 1957.

The authors have modified the preparation of a heretofore unstable iron reagent used for the determination of cholesterol in biological fluids. Instead of mixing the stock ferric chloride solution with sulfuric acid, the iron reagent is stabilized by dissolving ferric chloride in phosphoric acid. They report stability at room temperature for at least six to eight weeks. Standard error values for 15 total cholesterol assays and eight free cholesterol assays with iron reagent, were 7 and 3.7, respectively, and with Lieberman-Burchard reagent 8.9 and 4.3, respectively. Recovery studies performed by adding known amounts of cholesterol to serum gave no values less than 97 ± 7.9 per cent. E. COHEN.

Studies on Extrahepatic Cholesterol Synthesis and Equilibration in Man Using a Double Labeling Technique. G. V. LeRoy, R. G. Gould, D. M. Bergenstal, H. Wergin, and J. J. Kabara. *J. Lab. & Clin. Med.* 49: 858, 1957.

The authors describe a study to develop a double-isotope labeling technic for the investigation of the metabolism of sterols and steroid hormones in man. The present paper presents data on the relative rates of equilibrium of cholesterol between plasma and certain extra hepatic tissue.

Tritium-labeled cholesterol was fed to four patients for periods ranging from five to nine days at such a rate as to maintain an approximately constant plasma cholesterol specific activity. Tissue was then biopsied, the cholesterol isolated, and the specific activity used to obtain a rough estimate of the extent of equilibrium between plasma and tissue. Estimates of the relative rates of cholesterol synthesis in various tissues was made by the use of C^{14}-carboxy-labeled acetate.

Adrenal, ovary, and skin were shown to synthesize cholesterol rapidly from acetate. Differences between the specific activity in plasma and in tissues studied supported the concept that rates of equilibration vary widely with different tissues. K. R. CRISPELL

PROTEIN METABOLISM

The calculation of protein requirements for growth, maintenance of tissue metabolism, and probable fecal losses at various age levels, in relation to the biologic value of the ingested protein, provides a most interesting method by which to advance studies in the crucial field. Expansion of such investigations into areas in which vegetable protein provides the principal source of this dietary factor is eagerly awaited.

Theoretical Estimates of the Protein Requirements of Children. D. M. Hegsted. *J. Am. Dietet. A.* 33: 225, 1957.

In an excellent review of our current knowledge concerning factors which may be components of protein requirements for children and adults, the author poses the question as to whether a formulation of mixed vegetable proteins furnishes a practical way to meet protein requirements in underdeveloped areas. It is concluded on the basis of estimated minimal protein needs that these needs might easily be met by various vegetable mixtures, provided they are tolerated and suitably prepared.

The author lists estimated protein requirements for growth and maintenance for girls and for boys 0.5 month to 17.5 years of age. In preparing these estimates several assumed values were used. First of all, the growth requirements (g/kg/body weight/day) were calculated by multiplying the gain in grams per day by 0.18, assuming total tissue formed in growth contains 18 per cent protein.

The basal metabolism in calories per 24 hours multiplied by 12.5 was assumed as the requirement for maintenance. The requirement for growth plus that for maintenance gives the amount of absorbed protein needed. Addition of 10 per cent provides for fecal losses. When the biologic value of the protein under consideration is 100 per cent the dietary need equals the body needs. When the biologic value is lower the dietary need can be calculated as follows:

$$\frac{\text{estimated minimum} \times 100}{\text{biologic value}}.$$

On the basis of these calculations, the author estimates that the total protein requirement for girls may range from 2.31 g/kg at 0.5 month to 0.34 g/kg at 17.5 years. Similar figures for boys are 2.43 and 0.36. These are notably lower than amounts supplied to formula-fed infants and greater than is supplied by breast milk in amounts to supply the caloric needs under current infant feeding practices.

To test whether these estimated minimal protein needs might be supplied to the six-month-old child requiring 800 cal from individual vegetable sources, the author calculates the available protein in 800-cal portions of corn grits, white bread, white rice, and potato assuming biologic values of 54, 47, 75, and 71, respectively. The calculations revealed that these foods would fail to supply the daily protein needs by as much as 5.6 g/day in the case of white bread. Other deficits were 1.1, 5.8 and 5.4 g for corn grits, white rice and potatoes, respectively. For the one-year-old child requiring 975 cal the protein supplied

by the four foods would be in excess of its estimated minimal protein needs.

A plea is made for studies which will add to our understanding of the protein value of vegetable mixtures as few people exist on one food alone and the author feels that much could be done with available vegetable foods in areas where milk and other high quality protein sources are not available. J. M. SMITH

Protein Efficiency—Relative Nutritive Values of Proteins in Various Foods at Increasingly High Levels of Protein Intake. B. Sure. *J. Agric. and Food Chem.* 5:461, 1957.

The relative nutritive values of the proteins in nonfat milk solids, defatted dried whole eggs, soybean flour, wheat germ meal, cottonseed meal and corn gluten meal were determined in 15 to 30 per cent protein intake levels, using rats. "Protein Efficiency Ratio," gain in body weight per gram of protein intake, was the criterion for comparing nutritive values. The Protein Efficiency Ratios decreased as the level of protein intake was increased above 15 per cent with all foods. Protein Efficiency Ratios from lowest to highest at the 15 per cent protein intake level were—corn gluten meal 0.81, cottonseed meal 1.23, soybean flour 1.24, wheat germ 1.32, nonfat milk solids 1.40, defatted whole egg 1.51. The addition of 0.2 per cent L-lysine and 0.4 per cent DL-tryptophan to corn gluten meal at the 20 per cent level increased the Protein Efficiency Ratios only moderately. F. E. RICE

Supplementation of diets low in animal protein with certain amino acids, notably lysine, has been a subject of controversy. It has been claimed that nitrogen utilization and growth in children is enhanced by lysine additions. A similar action with respect to nitrogen retention is reported in adults; however, careful control of such experiments and longer observation periods are necessary before a satisfactory conclusion can be drawn.

Protein and Amino Acid Needs of the Aged in Health and Convalescence. A. A. Albanese, R. A. Higgons, L. A. Orto, and D. N. Zavattaro. *Geriatrics* 12:465, 1957.

The authors present a followup on the dietary habits of a large group of female patients past the age of 65. They report that it is possible, with a few exceptions, to maintain a good nutritional status on a daily minimum intake of approximately 54 ± 5 g of protein of which 39 per cent is derived from meat, in a self-selected diet of 1,560 calories. In a convalescent state, these patients tended to consume more protein and more calories on a self-selection basis.

Nitrogen balance studies demonstrated that about 50 per cent of convalescent patients were in negative nitrogen balance in spite of an adequate calorie intake. The addition of 600 to 900 mg of lysine to the diet seemed to increase the nitrogen balance. They conclude: "Our findings indicate that small additions of lysine to the diet of elderly adults under nutritiona, stress greatly enhance the biologic values of dietsl especially those low in meat proteins."

The length of control periods on four of the nine patients presented is not adequate for a good baseline. Also, it is known that convalescent patients may change from a negative to a positive nitrogen balance without any cause being determined. For this reason the studies using lysine supplements should be carried out with a double-blind study. K. R. CRISPELL

The immediate metabolic response to surgical procedures involves a loss of tissue protein of varying extent depending upon the type of operation performed. Mobilization of protein to supply calories during the period of low caloric intake occurring acutely at time of surgery occurs in an unexplained manner. The glucocorticoids of the adrenal cortex play a permissive role in this response which is reversed to some extent by increasing the caloric intake or by administration of anabolic hormones.

The Effect of Nutrition on Nitrogen Metabolism in the Surgical Patient. W. D. Holden, H. Krieger, S. Levey, and W. E. Abbott. *Ann. Surg.* 146:563, 1957.

Anabolic Effect of a New Synthetic Steroid on Nitrogen Metabolism after Operation. J. C. Peden, Jr., M. C. Maxwell, and A. Ohin. *A. M. A. Arch. Surg.* 75:625, 1957.

Among the metabolic changes which characteristically occur after injury is an increase in urinary nitrogen excretion. In general, the increase (intensity and duration) is directly related to the severity of the injury and the state of the individual at the time of injury. The metabolic upset is most marked in the previously healthy well nourished young adult male. W. D. Holden and his associates from a series of metabolic balance studies conducted during the past five years present additional evidence that the amount of urinary nitrogen excreted postoperatively is influenced by the patient's food intake. They found that the early loss of nitrogen and weight after mild operative trauma (hemorrhoidectomy, herniorrhaphy and "interval" appendectomy) by presumably well nourished men and women receiving only limited amounts of glucose solution as the exogeneous caloric source were similar to those observed in healthy, unoperated volunteers on similar nutrient intakes. In general, for comparable situations, the men lost greater amounts of nitrogen than the women. Further, the nitrogen and weight losses of patients (preoperative nutritional status not specifically described) undergoing more extensive, but fairly uniform, surgical trauma (gastric resection) were less in those subjects receiving postoperatively larger amounts of calories and nitrogen in the form of intravenous protein hydrolysates and hexose, or fat emulsion. Women again lost relatively less nitrogen than men. Decrease in postoperative urinary nitrogen excretion was also associated with the ad-

ministration of 250 mg of 19-nortestosterone cyclopentylpropionate, provided some exogenous nitrogen and calories were also given. The authors conclude: "The major portion of the nitrogen deficit reported to be the result of operative trauma is, in fact, the result of a poor nutritional intake." The authors do not imply that this situation obtains in the patient with severe accidental trauma.

Although Holden and his associates are of the opinion that the patients losing less nitrogen postoperatively are better off, this is difficult to prove.

Paden and his associates have also recently investigated the possible modification of postoperative urinary nitrogen loss. Their study centered around the effect of norethandrolone (17-α-ethyl-17-hydroxynorandrostenone; Nilevar®) given to 15 well nourished women undergoing elective abdominal total hysterectomy. These women received 76 g protein and 900 calories daily, intravenously, for the first three days postoperatively, thereafter orally. Five patients were given norethandrolone intramuscularly daily (25 or 50 mg) beginning immediately after operation and continuing for the first week; during the second week they received saline. Five other patients received their first injection of norethandrolone (25 or 50 mg) one or two days preoperatively and then daily for seven postoperative days. Five patients (control) received only saline intramuscularly for the first two weeks postoperatively. The net nitrogen loss of each of the control group was, on the average, about 20 g during the first week and about 18 g the second week. In contrast, patients receiving norethandrolone had net retentions of nitrogen of about 8 to 9 g each during the first postoperative week. During the second week patients receiving the steroid from the time of operation continued to retain about 8 g of nitrogen each, while those whose steroid therapy began preoperatively retained about 23 g. No differences in the clinical behavior of the patients in the various groups were noted; no virilizing effect of the drug was noted. However, there was significant sodium retention by the patients receiving norethandrolone; potassium balances were similar in all groups.

Two men with femoral fractures were also given norethandrolone. In one instance, this was begun while the patient was in negative nitrogen balance and in the other while in positive nitrogen balance (surprisingly, since this was in the first week postinjury); nitrogen and sodium retention increased in each patient.

No measurements were made by these authors as to the mechanism of the hormonal action nor to the fate of the "retained nitrogen." S. M. LEVENSON

Influence of Dietary Protein and Cortisone Acetate on Adrenalectomized Rats. R. C. Wolf. *Am. J. Physiol.* 190: 129, 1957.

Adrenalectomized immature rats were maintained for 20 days with 0.15 mg cortisone acetate when fed a 20 per cent casein diet. The effectiveness of the maintenance dosage of cortisone acetate (0.15 mg) was only slightly reduced when animals were fed a 5 per cent casein diet, but was ineffective when a protein-free diet was fed. Liver protein concentrations in normal and adrenalectomized rats were comparable. However, cortisone-maintained adrenalectomized rats had less liver glycogen and lipid than their respective dietary controls. Liver protein concentration varied directly with the percentage dietary protein while lipid and glycogen concentrations exhibited an inverse variation. AUTHOR

The protein requirement for maintenance of normal pancreatic function requires further study with particular respect to specific amino acid needs for enzymic synthesis and tissue repair.

Changes in Protein Metabolism in the Rat Pancreas on Stimulation. E. Farber and H. Sidransky. *J. Biol. Chem.* 222: 237, 1956.

The present study was undertaken to investigate the possible relationship between the protein-content of the pancreas and the secretion of pancreatic enzymes. White rats of both sexes were injected intramuscularly with aqueous pilocarpine (4 mg/100 g of body weight) or carbamylcholine (0.0125 mg), and sacrificed by a blow on the head at different time intervals after the injection. The pancreas was rapidly removed, chilled, and weighed. After the necessary treatment, the pancreas was analyzed for its amylolytic and proteolytic activity, as well as for its protein and nucleic acid content.

The results of these experiments indicate that the pancreatic weight and protein, as well as its proteolytic and amylolytic activity decreased after administration of carbamylcholine or pilocarpine. The total nucleic acid P, however, remained unchanged. The maximal loss in pancreatic protein after stimulation was about 20 per cent as compared to a 64 per cent loss of amylase activity. The rapidity of the fall of pancreatic enzyme activities and protein content was somewhat variable, but the levels were always low four hours after stimulation, and began to rise only after this time period. The rate of protein and amylase loss during the first four hours must, therefore, be greater than the rate of their resynthesis. After four hours there is a gradual return to control values, indicating an accelerated rate of protein synthesis. This was partially substantiated by means of experiments with biologically labeled amino acids.

This study does not support the hypothesis that pancreatic excretory enzymes have rapidly-synthesized, enzymatically inactive proteins as precursors.

M. K. HORWITT

Nitrogen release from liver slices as a method of evaluating the effect of various dietary programs upon protein metabolism may be a less cumbersome technic than per-

fusion of the isolated liver. The more physiologic state of the organ in the latter procedure is believed to provide for greater reliability than the liver-slice experiment.

Effect of Fasting on Protein Release by Liver Slices. E. Kaufman and E. Wertheimer. *Am. J. Physiol.* 190: 133, 1957.

The release of total nitrogen from rat tissue slices into an artificial medium was investigated under different experimental conditions. Protein was found to be the main nitrogeneous component released. Liver slices from fed rats release much less total nitrogen into the medium than those from fasted rats. This fasting effect is specific for liver tissue. A protein-free diet lowers total nitrogen release from liver slices, whereas a protein-rich diet raises it. Refeeding either the stock diet or a protein-free diet for one night after a five-day fast, lowers total nitrogen release to the level of that in fed animals. However, this is not the case after refeeding a low-ration diet. The fasting effect does not exist in rats fed a fat-rich, carbohydrate-free diet; it is lower than usual in rats fed a protein-rich, carbohydrate-free diet. The administration of glucose by stomach tube to fasted rats lowers total nitrogen release to normal fed levels in two hours.

AUTHORS

The Influence of Bodily Hydration on the Renal Concentrating Process. F. H. Epstein, C. R. Kleeman, and A. Hendrikx. *J. Clin. Investigation* 36: 629, 1957.

Studies on hydration were performed on normal young men. Various renal function and metabolic studies were performed which led to the results indicating that chronic underhydration, as well as prolonged forcing of fluids, drastically modifies the ability of the kidneys to reabsorb water and to concentrate solutes in the urine. It would appear that the state of hydration of tissues including, and perhaps most important, the cells of the distal and collecting tubules themselves may be the factors conditioning renal concentration.

S. O. WAIFE

The Effect of Feeding Protein and Urea on the Renal Concentrating Process. F. H. Epstein, C. R. Kleeman, S. Pursel, and A. Hendrikx. *J. Clin. Investigation* 36: 635, 1957.

Experiments were undertaken on normal young men to investigate the effects of alterations in dietary protein on the ability of normal kidneys to concentrate urine. It was found that the maximum renal concentrating capacity was altered strikingly by a change in the dietary intake of nitrogen, both as protein and (an unexpected finding) as preformed urea.

From this and the previous paper, it seems clear that variations in protein and urea excretion, as well as in the state of bodily hydration, produce well-marked and separable adaptive responses on the part of the renal tubules which have significance in terms of the body's economy of water.

S. O. WAIFE

Anemia may be due to protein deficiency in the presence of an adequate supply of iron and other hematinic factors. In hypoproteinemia, hemoglobin synthesis is impaired due to the diversion of essential amino acids to other areas of protein synthesis.

Diet and Anemia: Zymotic and Other Factors. B. S. Blatt and G. R. Wadsworth. *Proc. Brit. Nutrition Soc.* 15: 103, 1956.

The main part of this paper is concerned with the role of protein in maintaining normal amounts of hemoglobin in the blood. There is much evidence that an adequate supply of protein is necessary for normal hematopoiesis, but the idea that there is a prior claim for protein in the formation of new red cells has tended to draw attention away from protein deficiency as an important cause of anemia. The anemia that is associated with infection, trauma, and neoplasia might well be a manifestation of a diminished supply of protein available for hematopoiesis. The role of zymotic disease, of cancer and injury, in the development of anemia, especially in children, in pregnant and lactating women, and in subjects with some degree of protein malnutrition, is likely to be a most fertile field for badly needed further research.

B. SURE

Changes in Specific Blood Serum Protein Levels Associated with Parturition in the Bovine. B. L. Larson and K. A. Kendall. *J. Dairy Sc.* 40: 659, 1957.

Blood samples were taken from eight cows for considerable periods before and after parturition. Serum was prepared from the clotted blood by centrifugation. The serum samples were subjected to total protein and electrophoretic analysis which separated the serum proteins into eight distinct components. Beginning at about four weeks before and continuing until parturition there was a marked drop in serum protein levels, which was found to be due principally to losses in beta-2 and gamma-1 globulins and some alpha-globulins. The authors believe that as the immune globulins leave the blood stream they build up in the mammary gland, and later appear in the colostrum. Immune globulins have been demonstrated in the colostrum of certain animals other than the bovine, including the goat and rabbit, but not in the human.

F. E. RICE

Influence of a Protein-Free Diet on the Immediate and Latent Effects of Stilbestrol. S. R. Glasser. *Am. J. Physiol.* 189: 441, 1957.

The absence of protein from the diet of stilbestrol-treated rats modified, largely quantitatively, the response to the hormone. Stilbestrol, *per se*, induced a body weight loss which was greater in the protein-depleted than in the protein-fed animal. Recovery on an adequate protein diet after withdrawal of the hormone, permitted an immediate recouping of body

weight losses, whereas animals on the protein-deficient diet did not recover body weight losses. Stilbestrol administration increased excretion of nitrogen indirectly by restriction of food intake. This nitrogen loss was augmented by protein depletion. The marked retention of nitrogen by animals recovering on an adequate diet was more pronounced in the treated animal but rats continued on the protein-deficient diet maintained a negative nitrogen balance. The absence of protein from the diet diminished the anticatabolic influence of stilbestrol on total liver protein. Recovery on the basal diet allowed the repletion of liver protein but further decreases in liver protein concentration followed recovery on the deficient diet. A marked increase in the concentration of liver fat appeared concomitantly with the progressive depletion of liver protein in both treated and untreated groups. AUTHOR

VITAMIN A METABOLISM

Vitamin A is soluble in all fat solvents and is rapidly destroyed by oxidation, a process inhibited by such naturally occurring anti-oxidants as lecithin or tocopherol. The vitamin is necessary for cellular growth and to support integrity of epithelial structures. Deficiency of the vitamin interferes with the development of cartilage and endochondral bone growth. The role of this factor in intermediary metabolism has been studied in the following report which may clarify some of the earlier observations.

Studies on the Function of Vitamin A in Metabolism. G. Wolf, M. D. Lane, and B. C. Johnson. *J. Biol. Chem.* 225: 995, 1957.

Very little is known about the effects of vitamin A deficiency on intermediate metabolism. To study this further, weanling male albino rats were fed a synthetic diet deficient in vitamin A. Various C^{14} labeled carbohydrate intermediates were injected into animals with obvious deficiency as well as into pair-fed and *ad libitum* fed controls. No differences were seen in the specific labeling of CO_2, liver proteins, alanine, and aspartic acid using acetate-1-C^{14} in the deficient animals as compared to their pair-fed controls. Fatty acid and cholesterol had somewhat greater radioactivity in deficient animals which was considered to be due to a block of some metabolic pathway of acetate metabolism, but vitamin A is not considered essential for cholesterol biosynthesis. Tricarboxylic acid cycle reactions were not inhibited by vitamin A deficiency. On the other hand, incorporation of acetate-1-C^{14}, lactate-1-C^{14}, and glycerol-1-3-C^{14} into liver glycogen was greatly impaired in vitamin A-deficient rats, but utilization of glucose 1-C^{14} into glycogen and alanine was increased. Liver glycogen in deficient rats was practically nonexistent. These experiments indicate that vitamin A deficiency in rats leads to impairment of reverse glycolysis, or gluconeogenesis between the triose and glucose stages, but this may be secondary to changes in cell permeability or of hormone production. M. K. HORWITT

Action of Vitamin A on the Skin Following Intracutaneous Injection. H. A. Jewell, H. Taube, M. E. Nicholls, and R. A. Lehman. *Proc. Soc. Exper. Biol. & Med.* 96: 162, 1957.

New Zealand white male rabbits weighing 2 to 4 kg received intracutaneous injections of 0.03 ml of a solution of a commercial vitamin A palmitate (Keramin) equivalent to 50,000 U.S.P. units per ml. The injections were repeated daily for 28 days and the findings in the thoracic and abdominal skin were studied microscopically 1, 2, 4, 7, 14, 21 or 28 days after the last injection. The thickness of the epithelium was measured and mitoses were counted. One single application of the vitamin A preparation increased the thickness of all cell layers of the epidermis but the stratum corneum. The number of nuclei in the basal layer was slightly decreased, the nuclei in the spinous layer were significantly increased about four times the normal as early as after two days' treatment. However, there was no increase in mitoses. The occurrence of large young appearing nuclei in the absence of an increase in mitotic activity is interpreted as an indication that the maturation and developmental processes of cells of the stratum germinativum are slowed down by vitamin A.

M. SILBERBERG

Supplements of vitamin A during pregnancy have little effect upon the fetal serum levels of the vitamin. However, transfer of carotene across the placental membranes has been described. In the milk of humans or cows, the vitamin A content parellels that of the serum. Vitamin A-deficient diets result in a cessation of skeletal growth in young mammals, the effect of which is manifested in the nervous system due to improper development of the skull and vertebrae.

Vitamin A Deficiency in Infancy. M. H. Bass and J. Caplan. *J. Pediat.* 47: 690, 1955.

Two cases of vitamin A deficiency, a rare entity, are reported. Since a deficiency of this vitamin affects many organs, its manifestations are variable and may, therefore, be overlooked. The first case was that of a 10-month-old infant with generalized eczema who had been receiving a vegetable milk product with vitamins C and D since one month of age. Corneal ulcerations and blurring, albuminuria, hematuria, and hydrocephalus with a normal cerebrospinal fluid were present. Response was good to 25,000 units of vitamin A intramuscularly daily for six days and continued daily by mouth for two weeks.

The second case was that of a three-month-old infant with congenital absence of the bile ducts. As a result, the absorption of vitamin A, as well as other fat-soluble vitamins, was impaired. A tense, full fontanel was observed and studies of the CSF failed to reveal any chem-

ical or cellular abnormality. No ocular abnormalities were noted. The vitamin A content of the blood was only 34 units per 100 ml. Following large doses of vitamin A (50,000 units intramuscularly daily), the fontanel returned to normal. The authors discuss hydrocephalus, and quote Mellanby: "It is undoubted that changes of intracranial pressure are produced by vitamin A deficiency; the pressure of the cerebrospinal fluid in the cisterna magna is increased, a condition of slight but definite internal hydrocephalus is often found." Experimental studies in animals indicate that hydrocephalus is the result of retarded skull growth with normal growth of the nervous system.

The findings in cases reported when correlated with reports resulting from a review of the literature permit the following array of symptoms: (1) Retardation of mental and physical growth; (2) anemia with or without hepatosplenomegaly; (3) tendency to infection; (4) epithelial metaplasia. (a) In the eye: xerophthalmia and keratomalacia; (b) in the urinary tract: pyuria, hematuria, and excessive numbers of epithelial cells in the urine; (c) in the genital tract: cornified vaginal epithelium (d) in the respiratory and gastrointestinal tracts. (5) hydrocephalus with increased cerebrospinal pressure with or without isolated cranial nerve paralyses; (6) endocrine disturbance: gynecomastia.

The need for vitamin A therapy in infants on milk-free diets and with gall bladder disease is stressed. It is pointed out that evidence of increased intracranial pressure has also been observed in hypervitaminosis A and has been attributed to excessive production of cerebrospinal fluid by the chorid plexus. J. N. Ettel dorf

Vitamin A Levels in Idiopathic Hypercalcaemia. W. M. Fyfe. *Lancet* 1: 610, 1956.

The plasma levels of vitamin A, in the fasting state and four hours after a test dose of 12,500 i.u. per kg/body weight, were measured in eight children with "idiopathic hypercalcemia," five with marasmus, and 17 normal children. The levels, both fasting and after the test dose, were significantly higher in the hypercalcemic infants compared to the other two groups. It is suggested that vitamin A metabolism is disordered in these infants. F. E. Hytten

The accuracy of methods available for measuring vitamin A content in serum or plasma is highly variable due to great technical difficulties. This variability may account for the wide range of absorption demonstrated in the following experiments.

Absorption of Ingested Vitamin A. R. W. Hillman and N. H. Becker. *Gastroenterology* 32: 738, 1957.

Since vitamin A-tolerance tests are frequently employed in the diagnosis of faulty fat digestion, the authors have attempted to study the absorption of ingested vitamin A in normal adults.

Plasma concentration of vitamin A was determined immediately before and four hours after the ingestion of 150,000 i.u. of vitamin A in oil. In 50 subjects the absolute increase of plasma vitamin A concentration rose between −10 to +153 with an average of 28.9 ± 32.8 mcg per 100 ml. The mean relative increase (above the fasting level) was 46.1 ± 53.3 per cent. In a similar test using an aqueous preparation of vitamin A the mean absolute increase was 56.4 ± 20.9 mcg per 100 ml and the mean relative increase was 85.3 ± 11.6 per cent.

When similar tests were performed twice in each of the 50 subjects the mean difference between plasma responses in the duplicate tests in the same individuals was 22.6 ± 29.1 mcg per 100 ml or 45.8 ± 45.9 per cent using the oil preparation.

The data indicate a wide range of vitamin A absorption among normal persons and a marked variation in successive tests in the same individual. There was apparently no relationship between the fasting plasma vitamin A concentration and the response to a test dose of this substance. Variability in absorption seemed higher in the oil preparation of vitamin A than in the water-miscible preparation. These unpredictable differences may reflect changes in gastric emptying time.

The authors caution that impaired absorption should not be diagnosed on the basis of a single trial dose of vitamin A. J. B. Hammond

Vitamin A-Choline Interrelationship. R. Trasnin and R. F. Krause. *Proc. Soc. Exper. Biol. & Med.* 95: 574, 1957.

Vitamin A and choline seem to be interrelated in a physiologic sense. These authors determined the choline concentration of the liver of normal, vitamin A-deficient, and vitamin A-supplemented rats. Under the conditions of the study the choline concentration was highest in the livers of animals on a normal diet and with a normal level of vitamin A storage in the liver. Low concentrations of choline were found in livers which contained excessive or deficient amounts of vitamin A. The authors speculate that the low vitamin A blood levels found in many patients with malignant disease of the gastrointestinal tract in some way may be tied up with a disturbance of this choline-vitamin A relationship. S. O. Waife

The syndrome of hypervitaminosis A has become quite well-known to nutritionists. The symptoms include bone pain with hard, tender lumps in the extremities and cortical hyperostoses of the bones, hepatomegaly and jaundice, dry skin with loss of hair and cracking of lips. Increased secretion of cerebrospinal fluid and formation of renal glomerular filtrate has been observed in hypervitaminosis A.

Hypervitaminosis A. J. D. Pickup. *Arch. Dis. Child.* 31: 229, 1956.

Two cases are described of poisoning due to overdosage with a pure vitamin A preparation (avoleum).

A boy six years of age had had a daily intake of about 460,000 i.u. for at least six weeks, and a girl four years of age had taken about 350,000 i.u. for two years. Both were ill and febrile on admission, with facial swelling, extremely tender limbs, and enlarged livers. They are described in detail. The only significant biochemical finding was a high blood level of vitamin A. There was rapid improvement when the vitamin was stopped, but there were skin changes during recovery and the hair was shed. Only the girl showed bone changes; she had increased density at the growing ends of her long bones. The boy's liver was still palpable 10 months later, suggesting permanent damage. F. E. HYTTEN

Acute Hypervitaminosis A in Nursing Infants. J. Marie, G. See, and R. Sauvant. *Sem. Hôp. Paris.* 31: 251, 1955.

The massive ingestion of natural vitamin A (350,000 units) by nursing infants induces acute hydrocephalus, associated with hypersecretion and excessive pressure of cerebrospinal fluid.

The result, in the case of nursing infants, with open fontanels is the rapid development (24 hrs) of acute hydrocephalus after the ingestion of a massive dose of vitamin A. Profuse vomiting, a round projection from the fontanel and sometimes disjunction of the sutures take place. The syndrome has thus far always been mild. The amount of vitamin A reaches five times the initial amount six hours after ingestion begins, and begins to diminish at the 12th hour. Paradoxically, in dogs, it is frequently a deficiency of vitamin A which induces an excessive pressure of the cerebrospinal fluid. The authors have used the hypersecretive property of the cerebrospinal fluid (brought about by vitamin A) therapeutically in the treatment of hypotension of the cerebrospinal fluid in 100 nursing infants with acute meningitis who had undergone repeated lumbar punctures.

H. GOUNELLE

Influence of Cortisone on Teratogenic Effects of Hypervitaminosis-A. J. W. Miller and D. H. M. Woollam. *Brit. M. J.* 2: 196, 1957.

The effect of hypervitaminosis A in producing congenital brain and cranial defects in rats has been recently reported. Cortisone has also been shown to be teratogenic in rats. In the present experiments, briefly described, pregnant rats were given 60,000 i.u. of vitamin A daily from the 8th to the 13th day, 20 mg of cortisone acetate daily from the 9th to the 12th day, or both. With cortisone alone there were no gross malformations of the brain or calvaria, the incidence was 7.8 per cent with vitamin A alone and 36.6 per cent with both vitamin A and cortisone.

It is considered that cortisone potentiates, by some unknown mechanism, the teratogenic effect of hypervitaminosis A and that the mechanism may be applicable to man.

(Great caution should be used in translating these experiments into human terms. It is doubtful if these intakes of either vitamin A or cortisone have ever been approached by a pregnant woman. As pieces of isolated information which may one day help to finish the jig-saw puzzle of congenital anomalies in man these experiments may be useful, but they have no application at present.) F. E. HYTTEN

Effect of Cortisone on the Incidence of Cleft Palate Induced by Experimental Hypervitaminosis-A. D. H. M. Woollam and J. W. Miller. *Brit. M. J.* 2: 197, 1957.

In prior experiments similar to those described in this paper it was found that the incidence of cleft palate among the offspring of rats who were subjected to hypervitaminosis A during pregnancy was doubled if these mothers also received large doses of cortisone. "The results of the experiment suggest that cortisone acts by increasing the sensitivity of the developing tissues and thereby enhancing the teratogenic action of the hypervitaminosis-A." The application, if any, of this work to man is remote. F. E. HYTTEN

SODIUM AND WATER METABOLISM

The maintenance of normal serum sodium levels and fluid volumes depends upon multiple factors influencing the intake and excretion of these substances. With normal intake, the principal mechanisms involved in supporting homeostasis are those influencing renal tubular function. Some of these factors are discussed in the following abstracts.

The Renal Regulation of Water Balance. L. C. Welt. *Metabolism* 5: 395, 1956.

Water balance is maintained by the kidneys in response to changes in the osmotic composition of the body fluids which affect renal tubular function directly as well as indirectly through the rate of release of the antidiuretic hormone (ADH). The classical concept is that of passive diffusion of water with the active reabsorption of solute in the proximal tubule. This process permits approximately 15 per cent of the original volume of filtrate to reach the distal tubule in an iso-osmotic state. At this level under the influence of ADH, water may be actively absorbed or, in its absence, the abstraction of solute does not promote water reabsorption. There is evidence that an ADH-independent mechanism for water reabsorption exists at the most distal site of the tubule which is controlled by the rate of solute excretion. However, the conservation or rejection of water by the kidneys is dependent, in usual circumstances, upon the presence or absence of ADH. The stimulus for the formation of this hormone by the hypothalamic-hypophyseal system is the increasing osmolarity of the body fluids. Other conditions and agents promoting its secretion are painful stimuli, deficits in fluid volume, histamine nicotine, morphine and anesthetic agents. Ethyl alcohol is singularly able to decrease ADH production. The

effect of renal failure, edematous states, and endocrinologic disorders upon the response to ADH is discussed. C. R. SHUMAN

Electrolyte Changes in Renal Failure. J. P. Merrill. *Metabolism* 5: 419, 1956.

In renal failure electrolyte disorders may develop as a result of abnormal renal excretion or retention or as a consequence of metabolic disturbances affecting sodium and potassium transport. In chronic renal failure, the author, on the basis of pathologic and clinical findings, suggests that the destruction of a large number of nephrons would increase the solute load of the functioning renal tissue resulting in an osmotic diuresis. This produces polyuria and electrolyte losses. As the renal lesion progresses, the total volume of filtrate decreases resulting in oliguria, azotemia, and acidosis due to anion retention In the absence of cardiovascular failure, the administration of balanced sodium bicarbonate and chloride solutions may facilitate solute excretion by increasing solute load and by isotonic expansion of extracellular fluid volume increasing glomerular filtration rate. Potassium levels in serum may fall as a result of aldosterone hypersecretion or may rise in those with marked azotemia and acidosis. Hypocalcemia is believed to occur as absorption of ingested calcium is decreased from the gut. The rise in serum inorganic phosphate is also effective in depressing serum calcium levels. A rise in serum inorganic phosphate is also effective in depressing serum calcium levels. A rise in plasma magnesium and sulfate levels is observed, the latter contributing to the metabolic acidosis. The decreased bicarbonate levels regarded as compensatory phenomena prevented a severe decrease in pH.

In acute renal failure, hyponatremia may be observed as a result of sodium losses, dilution by excess water (intravenous fluids or endogenous fat catabolism), or the metabolic readjustment of cellular-extracellular fluid equilibrium. Potassium intoxication represents a dangerous complication accentuated by the development of acidosis. This condition should be combated by low potassium intakes, glucose and insulin, sodium and calcium containing tissues, and by intestinal irrigation or artificial kidney. C. R. SHUMAN

An important condition which has not been well recognized in the past is that of hyperosmolarity of the serum invariably associated with hypernatremia. This condition may be found in head injury due to a suppression of antidiuretic hormone release and decreased water intake. It has been described in patients receiving high protein feeding due to the increased levels of urine solute excretion with removal of large amounts of water from the body.

Hypernatremia. H. C. Knowles, Jr. *Metabolism* 5: 508, 1956.

Elevation of serum sodium levels has been described in conditions associated with cellular dehydration. The loss of water may result from combinations of inadequate intake, skin losses, deficient antidiuretic hormone or from obligatory urine flow from high solute loading. In diabetic acidosis, hypertonicity is frequently observed during the recovery period. This may be attributable to previous osmotic diuresis and administration of saline; in addition, treatment with insulin shifts water into cells with elevation of serum sodium concentrations. Hypernatremia was observed in patients with simple water deprivation associated with illness or abdominal surgery. Five of six patients receiving tube-feeding mixtures of high protein content died during periods of hypernatremia. The diuretic phase of acute renal failure and uncontrolled diabetes insipidus were found also to produce elevation of serum sodium due to excessive water losses. There may be no abnormal symptoms or signs in mild cases while severe cases may manifest coma or bizarre neurologic signs. Therapy consists of the administration of water and withholding salt while guarding against water intoxication and salt depletion. The prognosis in prolonged hypertonicity is poor despite proper therapy; mild cases usually recover uneventfully. C. R. SHUMAN

Hyponatremia is a relatively common electrolyte disturbance of multiple etiology. In the majority, the reduced serum levels are observed to be the result of dilution rather than depletion of serum sodium. The clinical status of such patients may be aggravated by administration of sodium.

The Pathogenesis of Hyponatremia: Physiologic and Therapeutic Implications. I. S. Edelman. *Metabolism* 5: 500, 1956.

The presence of hyponatremia does not always signify the presence of sodium depletion as determined by the application of isotopic methods. In conditions associated with excessive salt losses such as renal disease, diarrhea or sweating, there is sodium depletion. In patients with edema, hyponatremia coexists with an excessive body sodium content. The condition of low serum sodium may develop as a result of intracellular osmolarity; this may occur with potassium depletion or reduction of intracellular anionic composition. In these instances, hyponatremia may follow because of migration of sodium into cells or migration of water from cells. The author comments that the presence of edema signifies increased body sodium and that depletion of body sodium in patients with congestive failure without renal disease is rare. The administration of saline to the edematous, hyponatremic patient will further distort body composition and may produce pulmonary edema. Sodium deficits are replaced in patients with dehydration and hyponatremia. The formula for estimating sodium deficits is: sodium deficit = (140-serum sodium) x total body water. In those with hypopotassemia, the administration of potassium at the rate of 100 to 250 meq/day will often raise the serum sodium levels. The majority of edematous hyponatremic pa-

tients will respond to deprivation of salt and water. The water intake is limited to 1,200 ml/day. The limiting factor is that of thirst which may occur when serum sodium is low due to intracellular hypo-osmolarity. In certain patients delirium or coma may require hypertonic saline administration and water deprivation.

C. R. SHUMAN

Chronic sodium chloride administration produces a severe hypertensive state in experimental animals. The sodium levels in tissues, particularly in arteries, has been shown to be elevated in hypertensive animals and humans. Individuals with hypertension ingest larger amounts of salt than non-hypertensive subjects. The experimental and clinical data support the view that high levels of salt intake may produce hypertensive disease.

Etiological Role of Sodium Chloride Intake in Essential Hypertension in Humans. L. K. Dahl and R. A. Love. *J.A.M.A.* 164: 397, 1957.

A group of 1,346 adults, members of the Brookhaven laboratory staff reporting for an annual physical examination, were classified according to their habitual salt intake and blood pressure. Of this group of patients, 105 were classified as hypertensive (defined as a pressure above 140/90 mm Hg). These subjects were not distributed at random. There were 61 among the 581 persons with a *high*-salt intake, which was defined as a person routinely adding salt to the foods before tasting them. Forty-three of the hypertensive subjects were among the 630 with an *average* salt intake, which is defined as adding salt to food if, after tasting, it is found to be insufficiently salty for the taste. Only one hypertensive was found among the 135 with a *low*-salt intake, which was defined as a group denying the adding of salt to the diet. Statistical analysis of this distribution shows that it could have occurred by chance in less than one of a thousand instances.

A further distribution of the patients on the basis of their weight suggests that those who are not overweight but are on a high-salt diet have several times the incidence of hypertension found among similar individuals on a low-salt intake. The highest incidence of hypertension was found among those who are both overweight and have a high-salt diet.

The authors point out the several limitations of this type of study and submit the hypothesis that the level of sodium intake is a primary etiologic factor in the development of essential hypertension.

This observation is of interest in view of the recent finding that among natives in the West Indies, who happen to ingest a very high-salt intake, the incidence of hypertension is higher than would be expected on the basis of the usual racial factors. S. O. WAIFE

Chronic Sodium Chloride Toxicity in the Albino Rat. III. Maturity Characteristics, Survivorship, and Organ Weights. R. G. Tucker, C. O. T. Ball, W. J. Darby, W. R. Early, R. C. Kory, J. B. Youmans, and G. R Meneely. *J. Gerontol.* 12: 182, 1957.

Male Sprague-Dawley rats, five weeks of age, were fed purified diets containing nine different levels of NaCl varying from 0.01–21.0 per cent. The diet containing 0.15 per cent NaCl was considered the control ration. Rats kept on the control diet were larger, attained higher weights, and lived longer than those fed diets high or low in NaCl. Control animals showed weight increase for a longer period of time than rats fed diets containing 7 per cent or more NaCl. The systolic blood pressure taken at the time of maximal weights and the electrocardiographic changes correlated directly to the NaCl content of the diet. The changes in the myocardium indicated muscle damage and hypertrophy and were similar to those seen in hypertensive patients.

M. SILBERBERG

Rigid sodium restriction enhances the activity of the adrenal zona glomerulosa, the site of formation of aldosterone. It is of interest that several of the reported cases of aldosteronoma have been maintained for five years on sodium-restricted diets.

Alterations in the Rat Adrenal Cortex Induced by Sodium Deficiency: Steroid Hormone Secretion. A. B. Eisenstein and P. M. Hartroft. *Endocrinology* 60: 634, 1957.

Alterations in the Rat Adrenal Cortex Induced by Sodium Deficiency: Correlation of Histologic Changes with Steroid Hormone Secretion. P. M. Hartroft and A. B. Eisenstein. *Endocrinology* 60: 641, 1957.

These papers present the results of experiments to determine the effect of rigid sodium depletion on the rat adrenal cortex. Young, male rats were placed on a synthetic diet devoid of sodium and drank distilled water. Pair-fed control rats were treated identically except for addition of sodium chloride to the diet. Animals from each group were sacrificed at frequent intervals up to 60 days, and body weight, adrenal weight, serum electrolytes, and blood hematocrit were determined. One adrenal from each rat was used to determine the ability of the isolated gland to secrete steroid hormones and the other adrenal was used for histologic study.

Progressive, marked enlargement of the zona glomerulosa of sodium-deficient rats was observed and correlated with a major increase in the secretion of aldosterone by the isolated adrenal glands. As the zona glomerulosa became hyperplastic, there was atrophy of the zona fasciculata and concomitant reduction in the secretion of corticosterone and an unidentified steroid. As a result of reduced secretion of corticosterone and the unidentified steroid, total synthesis of biologically active hormones by isolated adrenals of sodium-depleted rats was significantly diminished when compared to that of pair-fed controls.

The authors believe that atrophy of the zona fasciculata and decreased corticosterone secretion occurred secondary to marked enlargement of the zona glomeru-

losa with increased production of aldosterone. It is also felt that these experiments provide evidence to suggest that factors other than ACTH may influence adrenocortical function. A. B. EISENSTEIN

Barrier Offered by Gastric Mucosa of Healthy Persons to Absorption of Sodium. R. J. Reitemeier, C. F. Code, and A. L. Orvis. *J. Appl. Physiol.* 10: 21, 1957.

Isotopically labeled water and sodium were swallowed by healthy adults in a modified head-down position. Absorption rates were determined by integrating the appearance rates in arterial blood during absorption with rates of disappearance. These disappearance rates from arterial blood were determined in control periods after rapid intravenous injection. In nine of eleven tests it was observed that the stomach contained isotopes for 2 to 32 minutes. During this time arterial blood contained D_2O but not radiosodium. After the period indicated sodium began to appear slowly in blood. Once isotopes entered the small intestine absorption was rapid. Slow gastric absorption of radiosodium possibly occurred via antral mucosa while the portion of the gastric mucosa which secretes acid behaves as a barrier to sodium absorption. M. J. OPPENHEIMER

Comparison of Rate of Absorption of Labeled Sodium and Water From Upper Small Intestine of Healthy Human Beings. R. J. Reitemeier, C. F. Code, and A. L. Orvis. *J. Appl. Physiol.* 10: 256, 1957.

Isotopically labeled water and sodium were introduced into the third portion of the duodenum via a tube. Observations were made on the rate of appearance of the isotopes in arterial blood. The data were obtained by rapid frequent sampling. Rate of passage from intestinal lumen to blood stream was also determined by calculation. Water was absorbed more rapidly than sodium. Mean initial rate of absorption of deuterium was 20 per cent/min of the administered and for radiosodium 10 per cent. Two-thirds of the deuterium was absorbed in seven minutes. It required ten minutes to absorb the same percentage of radiosodium.
M. J. OPPENHEIMER

The following discussion describes a vascular lesion in experimental animals which appears in lathyrism and resembles that of vitamin E depletion. However, the muscle changes found in tocopherol deficiency have not been found in lathyrism so that a clear relationship between these two conditions remains to be established.

Histopathology of Aortic Aneurysms in the Lathyrus-Fed Rat. D. G. Walker and Z. T. Wirtschafter. *Arch. Path.* 61: 125, 1956.

A diet containing 50 per cent of the sweet pea *Lathyrus odoratus* was fed to weanling rats for periods up to six weeks. The animals developed dissecting aneurysms of the aorta and aneurysms of the pulmonary and coronary arteries. The histologic changes consisting of destruction of elastic fibers (elastinolysis) and fibroblastosis are considered to be the expression of a general metabolic disorder. The findings resemble in some respects those observed in vitamin E deficiency. M. SILBERBERG

ABSORPTION OF FAT

Ingested fats are absorbed after hydrolysis by the action of lipase, most of which is derived from pancreatic secretions. Bile acids participate as emulsifying agents in reducing surface tension and increasing solubility of fats and fatty acids. The bile salt-fatty acid complexes enter the intestinal mucosa where triglycerides or phospholipids are formed to be extended into the lymphatic system and portal circuit. The rate of absorption is increased at higher doses of fat feeding. Other factors influencing absorbability are melting point, hydrogenation, chemical state, and the functional status of the intestine.

The Digestion and Absorption of Fat in Dog and Man. L. K. Knoebel and E. S. Nasset. *J. Nutrition* 11: 405, 1957.

Despite a century of investigation, the mechanisms of fat digestion and absorption are not fully understood. The purpose of the present work was to investigate in more detail the composition of fat in the stomach and in different regions of the small intestine of the dog and man after feeding various fat test meals. It was also hoped that a better understanding of the absorptive processes taking place would be gained by making comparisons between these studies *in vivo* and certain investigations performed by other workers *in vitro*.

Small amounts of endogenous lipid of fairly constant composition were recovered from all parts of the small intestine of dogs fed fat-free test meals. When cottonseed oil was fed, the composition of lipid recovered from the small intestine of dogs killed after three hours closely resembled that reported by others during the first one to two hours of the hydrolysis of triolein *in vitro*. The composition of lipid recovered from jejunal fistula dogs fed cottonseed oil was similar to that reported by others as being produced during the first one-fourth to one-half hour of the hydrolysis of triolein *in vitro*.

Fat recovered from fistula dogs fed diglycerides was very similar in composition to that produced during the first one-half hour of the hydrolysis of diolein *in vitro*. Fat may be hydrolyzed, therefore, in much the same manner *in vivo* as *in vitro*. Hydrolysis of monoglycerides fed to dogs was very rapid in the duodenum. The proportion of diglycerides and triglycerides was greatly increased in the intestine, suggesting that synthesis and hydrolysis occur simultaneously.

Gastric lipolysis of cottonseed oil and monoglycerides in the dog was slight, while butterfat was somewhat more digested in the stomach of humans. The composition

of lipid recovered from the small intestine of man fed butterfat triglycerides was very similar to that found in the small gut of dogs fed cottonseed oil. B. SURE

The Absorbability of Natural and Modified Fats. D. H. Galloway, G. W. Kurtz, J. J. McMullen, and L. V. Thomas. *Food Res.* 21: 621, 1956.

The natural fats and oils tested were butterfat, lard, and the oils of cottonseed, soybean, corn, palm, and coconut. Modifications of these fats used in the experiment were (a) complete hydrogenation to convert unsaturated to saturated fatty acids, (b) lard fat in which the glycerol radical was substituted by mannitol and (c) substitution of one-third of the fatty acid radicals by a butyryl group. Mature rats were fed diets containing 20 per cent fat in each test and fat absorbability (digestibility) was determined from feces analyses.

It was found that hydrogenation greatly reduced the digestibility of all fats except coconut oil. The digestibility of butter fat was reduced by hydrogenation from 97.8 per cent to 61.0 per cent. The losses in digestibility of the five other fats and oils were still greater. Absorbability (digestibility) was observed to have an inverse relationship to the content of the natural and modified fats in saturated fatty acids, 18-carbon chain and above. This is due in part to the higher melting points of the long-chain saturated fats. The substitution of mannitol for glycerol in the fat molecule made no difference in digestibility but the introduction of the butyryl radical in the fat molecule as a replacement for one of the long chain fatty acid radicals resulted in an increase in the digestibility of the fat. It was concluded that digestibility depends primarily on the amounts and chain length of the saturated fatty acids, and their arrangement within the glyceride structure.

F. E. RICE

Absorption of Monoglycerides. H. C. Tidwell. *Am. J. Physiol.* 189: 537, 1957.

The rate of absorption of glyceryl monooleate, when fed alone, appeared to be much slower than that of other glycerides. However, the monooleate was found to be relatively unavailable for absorption because a large part of it was retained for a time in the stomach as a thick gel. In contrast, the chylomicronemia was markedly greater after feeding olive oil, Tween 80, or 1:1 mixtures of the glyceryl monooleate and olive oil or the fatty acids prepared from it. The slower absorption of the monoglyceride was not associated with a decreased lipid retention over a longer period, because eventually all of the lipid became available for absorption. In an unsuccessful attempt to prevent excessive gastric retention of this lipid, several 10 per cent emulsions of the monooleate were employed. Observation showed that the retention in these attempts could not be attributed solely to gel formation, but more likely to a decreased gastric motility. Nevertheless, a similar rate of absorption of the monooleate and olive oil was obtained if only that part of the lipid were considered which had passed from the stomach and was therefore readily available for absorption. Insufficient evidence is at hand to determine whether the major hydrolysis of the monoglycerides, which is necessary for formation of the triglycerides of the chyle, occurs in the lumen or the mucosal cell. AUTHOR

Impairment of Triglyceride Absorption by the Exclusion of Pancreatic Juice in the Rat. E. Karvinen, T. M. Lin, and A. C. Ivy. *Am. J. Physiol.* 188: 61, 1957.

A fat balance study was conducted on 12 rats with and 12 rats without exclusion of the pancreatic juice, using tripalmitin, trielaidin, triolein, tallow and oil, or fats varying in regard to melting point, saturation, and *cis* and *trans* isomerism. Exclusion of pancreatic juice decreased the utilization of these fats. The decrement ranged from 14 to 18 per cent of the intake of the different fats when fed at a level of 8 per cent of the dry weight of the diet. The extent of impairment expressed as millimols could not be correlated with the physical and chemical characteristics of the fats; but if expressed as percentage of fat absorbed, the impairment was related to the melting point of the fat. The fecal elimination of soap was increased significantly by the exclusion of pancreatic juice in the case of corn oil, triolein, and tallow; was not significantly increased in the case of trielaidin; and was decreased in the case of tripalmitin, due probably to decreased hydrolysis. Exclusion of pancreatic juice increased the elimination of endogenous total lipid and of soap. Fat utilization was correlated more closely with the melting point of the fats than with their saturation, suggesting that the melting point is concerned in determining utilization more than any other characteristic of the fat. AUTHORS

A useful method for studying fat absorption is that of measurement of levels of radioactivity in the lipid fraction of blood following oral administration of I^{131}-labeled triolein. Separation of the label from the tagged fat in the intestine may introduce inaccuracies if whole serum is used in this study.

The Uses of I^{131} Triolein in the Study of Absorptive Disorders in Man. P. Beres, J. Wenger, and J. B. Kirsner. *Gastroenterology* 32: 1, 1957.

I^{131}-labeled triolein was administered as part of a test meal to 30 normal subjects and to 27 patients with various types of absorptive defects. The levels of radioactivity in the lipid fraction of the blood, and the amount of radioactive material excreted in the urine and in the feces following the test meal were measured.

In the normal group, radioactivity of the lipid fraction of the blood rose rapidly to a peak between four and six hours after ingestion of the test meal—the levels varying between $2^1/_2$ and 8 per cent of the dose and averaging $5^1/_2$ per cent. In marked contrast, five

patients with sprue showed levels of less than one-half of 1 per cent of the dose after eight hours. Therapy with prednisone increased the absorption to near normal levels in three cases. Patients with chronic pancreatitis, carcinoma of the pancreas, biliary obstruction, and postgastrectomy steatorrhea showed variable degrees of impairment of absorption. On the other hand, patients with regional enteritis with steatorrhea, as demonstrated by chemical fat studies of the stool, showed normal or near normal levels of absorption.

In five normal subjects, radioactivity of the feces collected from 72 to 96 hours following a test meal varied from 0.2 to 1.0 per cent of the test meal. Variations in the radioactivity of the feces paralleled the degree of absorptive defect. Radioactivity of the urine during the first eight hours following ingestion of the test meal paralleled the blood levels, but did not appear to add any additional information.

It is concluded that this method offers an accurate and convenient method of studying fat absorption in clinical disorders except that due to regional enteritis.

It is of interest that the levels of radioactivity of the lipid fraction of the serum correlated well with the degree of fat absorption, in contrast with the results obtained by McKenna *et al.* who tested the radioactivity of the whole serum. J. B. HAMMOND

The Use of I^{131} Labeled Fat in the Study of Fat Digestion and Absorption in Normal Individuals and in Patients with Disease of Fat Absorption. R. D. McKenna, R. H. Bourne, and A. Matzko. *Gastroenterology* 32: 17, 1957.

Glycerol trioleate in olive oil labeled with I^{131} was administered as a test meal to 15 normal subjects, seven with impaired absorption of fat, and three with partial gastrectomy. The levels of radioactivity of the blood serum at 1, 2, 3, 4, and 6 hours after the test meal, of the 48- to 72-hour stool specimen, and of 24-hour specimens of urine were determined.

The peak serum levels of radioactivity in the normal group occurred three to four hours after the test meal was administered. Patients with steatorrhea as determined by chemical fat analysis of the stools showed decreased blood serum levels of radioactivity and higher levels in the feces as compared to normals. When blood serum levels were compared, there was some overlapping between the two groups, but the stool studies accurately differentiated patients with normal fat absorption from those without. Normal subjects excreted 4 per cent or less of the ingested dose, while those with steatorrhea excreted 12.2 to 39 per cent of the ingested dose. J. B. HAMMOND

As in the studies with calcium, it has been shown that fatty acids are excreted into the intestinal lumen from the mucosal cells. The elimination of pancreatic juice increases the elimination of endogenous lipid and calcium soaps.

Metabolism of Endogenous Lipid of the Intestine. K. S. Kim, J. L. Bollman, and J. H. Grindlay. *Am. J. Physiol.* 184: 445, 1956.

Total fatty-acid output in the intestinal lymph in rats and in the thoracic-duct lymph in dogs was studied during fasting and during a fat-free diet period. Daily total fatty-acid output in the intestinal lymph was found to be fairly constant during fasting or when the rat was fed a fat-free diet. There is an inverse relationship between total fatty-acid concentration and the volume of intestinal lymph. In the absence of pancreatic juice or of both bile and pancreatic juice, the total fatty-acid output in the thoracic-duct lymph in dogs was decreased to one-third or one-fourth that of the normal dog. The endogenous lipid metabolism of the intestine is discussed in relation to steatorrhea. AUTHORS

Exchange of Free Fatty Acids and Glyceride Fatty Acids During Fat Ingestion in the Human Intestine. E. H. Ahrens, Jr. and B. Borgstrom. *J. Biol. Chem.* 219: 665, 1956.

Previous studies have shown that in the rat intestine, hydrolysis to fatty acids and glycerol took place with preferential production, of 1,2-diglycerides and 2-monoglycerides. Two patients without evidence of gastrointestinal dysfunction were fed test meals containing C^{13} labeled palmitic or oleic acids suspended in triolein, and the duodenal and jejunal contents were aspirated for several hours. Lipids were extracted, separated by countercurrent and other technics, and free fatty acids, mono-, di-, and triglycerides were quantitatively determined with their specific C^{13} labeling. Highest glyceride labeling was found in the diclass, followed by tri- and monoglycerides. Intestinal synthesis of new esters was shown to occur along with hydrolysis, but the over-all reaction is toward hydrolysis, since resynthesis of monoglycerides from glycerol and fatty acids does not occur in the intestine. Several pathways of synthesis and degradation of glycerides are postulated, with some evidence being presented that free acids are more rapidly absorbed than glycerides, but that fatty acids are liberated more rapidly than the rate of absorption. M. K. HORWITT

Pancreatic lipase is involved only in the hydrolysis of glyceride-fatty acid compounds and not in the absorption of the fatty acids released after digestion.

Function of Pancreatic Juice in Fat Utilization in the Rat. E. Karvinen, T. Lin, and A. Ivy. *Am. J. Physiol.* 189: 113, 1957.

Tripalmitin, a saturated, and triolein, an unsaturated fat, and their respective fatty acids were fed at a level of 8 per cent by dry weight of the diet to 12 sham operated control and 12 pancreatic duct ligated rats. It was found that exclusion of pancreatic juice significantly decreased the utilization of both triolein and tripalmitin. Thus, the pancreatic juice is essential for the utilization of both saturated and unsaturated fat. It

was found also that exclusion of pancreatic juice did not influence the absorption of oleic and palmitic acid. Therefore, it is concluded that pancreatic alkali does not exert any significant effect on the absorption of fatty acid in the amounts fed whether saturated or unsaturated, and that pancreatic lipase and/or the facilitation by pancreatic alkali of the hydrolysis of fat by any lipase present in the intestine is the factor (or factors) in pancreatic juice which is essential for the complete utilization of both saturated and unsaturated fat. The observed decrease in the utilization of tripalmitin produced by the exclusion of pancreatic juice was not larger than the decrease in the utilization of triolein; therefore, it is concluded that a desaturation of fat by a pancreatic dehydrogenase is not essential for the absorption of saturated fat. AUTHORS

Impairment of fat absorption in malnutrition has been described in many earlier studies. The mechanism has not been elucidated; there may be an abnormality in fat-splitting or a disturbance in absorptive capacity of the intestinal mucosa. The favorable response observed after treatment with skim milk and nutritional repair suggests impairment of pancreatic and intestinal function as the cause of the disturbance.

Fat Absorption in Chronic Severe Malnutrition in Children. F. Gómez, R. R. Galván, J. Cravioto, S. Frenk, J. V. Santaella, and C. de la Peña. *Lancet* 2: 121, 1956.

The fat balance was studied for four-day periods in 14 children of unstated ages, suffering from severe chronic malnutrition. The percentage fat absorption was low in all cases on admission to hospital, although often without manifest steatorrhea. It improved during treatment and reached normal levels after recovery.

Although there is a defect of fat absorption, the percentage absorbed was not affected by the amount ingested; so that an increased amount of dietary fat gives a proportionately greater net absorption.

F. E. HYTTEN

ALDOSTERONE AND POTASSIUM METABOLISM

The presence of a potent sodium-retaining factor in the urine of edematous patients was demonstrated by Deming and Luetscher in 1950. The active mineralocorticoid was isolated in 1952; it was crystallized and identified in 1954. This hormone promotes the loss of potassium from cells and increases its renal excretion. Renal retention of sodium and hydrogen ions occurs under its influence. The net result is the production of hypokalemic alkalosis, intracellular acidosis and an alkaline urine.

The Role of Aldosterone in Normal Homeostasis and in Certain Disease States. F. C. Bartter. *Metabolism* 5: 369, 1956.

Aldosterone has been isolated from the adrenal gland and has been detected in blood and urine in normal and diseased subjects. It is a highly potent compound in control of renal sodium conservation and potassium excretion having an action about 30 times that of desoxycorticosterone. Its effects upon eosinophil depression and carbohydrate metabolism are negligible. The physiologic stimuli to secretion of aldosterone have been found to be sodium deprivation, changes in extracellular volume, and potassium loading. The author presents evidence supporting the view that changes in extracellular volume are fundamental in control of aldosterone production, a contraction of ECF extracellular fluid volume producing the stimulus for increased secretion and vise versa. The control exerted by ACTH over aldosterone secretion is less significant. In hypopituitrism there is a decrease in secretion which is raised by ACTH administration.

Aldosterone secretion is raised secondarily in cirrhosis, nephrosis, cardiac failure and toxemia of pregnancy by an unknown mechanism. Possibly the location of the abnormally retained fluids is such that it does not operate in aldosterone control. The effect is that of increasing sodium and water retention. In renal disease associated with sodium-wasting, aldosterone secretion is raised resulting in renal excretion of potassium and alkalosis. Primary hyperaldosteronism occurs in certain patients with adrenal tumors. The measurement of urinary sodium excretion on low sodium intake will aid in the differentiation of the latter conditions. C. R. SHUMAN

Postulated mechanisms responsible for increased aldosterone secretion are stated in the above abstract. Further evidence is cited to support the view that changes in fluid volume influence receptors which directly alter the rate of release of aldosterone.

Ascites Formation Without Sodium Intake in Dogs With Thoracic Inferior Vena Cava Constriction and in Dogs With Right Sided Congestive Heart Failure. W. C. Ball, Jr., J. O. Davis, and M. J. Goodkind. *Am. J. Physiol.* 188: 578, 1957.

Dogs were subjected to constriction of the thoracic inferior vena cava or main pulmonary artery and were deprived of food for a period of four days thereafter. In association with a high venous pressure, 60 per cent of the dogs formed ascites despite the absence of Na intake. Measurements of T-1824 dye space provided evidence that plasma volume was not increased. An increase in urinary excretion of aldosterone was associated with a markedly reduced urinary Na excretion. The data are interpreted as supporting the concept that elevated venous pressure initiates edema formation in congestive heart failure and that renal Na retention occurs as a secondary phenomenon. AUTHORS

In primary aldosteronism, severe potassium deficiency has been a consistent feature. In this condition, as well as other clinical states associated with potassium depletion, renal functional impairment, degenerative tubular lesions and pyelonephritis have been described frequently. Experimental data have accumulated in support of these findings.

Histochemical Study of the Kidney of Rats Fed Diets Deficient in Potassium. J. M. Craig and R. Schwartz. *A.M.A. Arch. Pathol.* 64: 255, 1957.

Sprague-Dawley rats weighing about 125 g were fed diets deficient in both sodium and potassium, or in sodium or potassium alone, or in potassium alone and restricted in caloric intake to the amount of food consumed by the animals fed a sodium- or potassium-free diet. The potassium-deficient rats showed renal change such as tubular damage associated with tubular hyperplasia and hypertrophy. These changes were most marked in the medulla and in the terminal collecting tubules. The interstitial tissue contained PAS-positive material containing macrophages. Acid phosphatase activity in the second portion of the collecting tubules was strikingly increased. The tissue changes were intensified by diets deficient in both potassium and sodium. Under the latter conditions, lymphocytic infiltration was also noted in the cortex in addition to the aforementioned tubular lesions. M. SILBERBERG

Defect in the Renal Tubular Reabsorption of Water Associated With Potassium Depletion in Rats. W. Hollander, Jr., R. W. Winters, T. F. Williams, J. Bradley, J. Oliver, and L. G. Welt. *Am. J. Physiol.* 189: 557, 1957.

The effect of graded degrees of K depletion on the ability to produce a concentrated urine was studied in Sprague-Dawley rats. With increasing degrees of K depletion, as measured by the concentration of K in fat-free skeletal muscle, there was a progressive decrease in the maximum urinary concentration. This defect of the renal concentrating mechanism appeared to be better correlated with the degree than with the duration of potassium depletion and could be demonstrated either by the use of exogenous vasopressin or by water deprivation. The potassium-deficient rats in at least one experiment developed a significant polydipsia. The data do not allow any conclusions with respect to the relationship of the polydipsia to the renal concentrating defect except that the latter at least was not severe at the onset of the increased water intake. AUTHORS

Restriction of sodium intake is capable of modifying the cellular changes associated with potassium depletion. The reduced availability of sodium to replace intracellular potassium or the maintenance of normal sodium/potassium ratio may prevent the development of cellular damage.

Electrolyte Imbalance and Intracellular Potassium Exchange. M. H. Rahman, L. E. Frazier, R. H. Hughes, and P. R. Cannon. *A.M.A. Arch. Pathol.* 63: 154, 1957.

During periods up to one year young albino rats of the Sprague-Dawley strain were fed purified diets containing (1) normal levels of potassium and sodium, (2) low levels of both substances (3) low levels of potassium and high levels of sodium, (4) low levels of potassium and very high levels of sodium, (5) normal levels of potassium and high levels of sodium. Diets high in sodium chloride but low in potassium markedly increased urinary excretion of potassium and induced focal myocardial necrosis characteristic of potassium deficiency. These lesions were absent or limited in animals fed diets low in sodium chloride. It seems as if imbalances between sodium and potassium lead to predominance of sodium ions within the cells, to intracellular displacement of potassium and subsequent potassium deficiency M. SILBERBERG

Sodium and Potassium Concentrations in the Saliva of Normotensive and Hypertensive Subjects. W. Neidermeier, S. Dreizen, R. E. Stone, and T. D. Spies. *Oral Surg., Oral Med., Oral Path.* 9: 426, 1956.

The average sodium concentration in the saliva of 37 untreated essential hypertensive patients was significantly lower than the average value for 42 normotensive subjects of the same age. This difference was true both for unstimulated and stimulated saliva. Stimulated saliva contained on the average twice as much sodium as unstimulated saliva. In contrast, the level of potassium in saliva and the rate of flow were virtually the same in both groups. Stimulated saliva contained on the average 25 per cent less potassium than unstimulated. The rate of flow was two to three times as great after stimulation. The daily diets of two groups of six normotensive and six hypertensive subjects were supplemented with known amounts of sodium chloride or potassium chloride. No significant influence on sodium and potassium contents was produced in either normal or hypertensive patients.

The difference in salivary sodium values between normal and hypertensive individuals, between unstimulated and stimulated samples, and the lack of influence of increased dietary sodium form an interesting basis for postulating an intimate neural control of sodium secretion in the saliva. J. H. SHAW

ITEMS OF GENERAL INTEREST

Differential Serum Vitamin B_{12} Concentrations in Mothers and Infants. W. P. Boger, G. M. Boyne, L. D. Wright, and G. D. Beck. *New England J. Med.* 256: 1085, 1957.

The vitamin B_{12} content of blood samples of mothers at parturition was compared to the vitamin B_{12} content

of blood obtained from the umbilical cords of their infants. The average serum concentration of vitamin B_{12} of the infants was approximately twice as high as that of the mothers. M. W. BATES

Lactation and Heredity. I. G. Wickes and M. P. Curwen. *Brit. M. J.* 2:381, 1957.

There is no information about the effect of heredity in human lactation; in cattle there is, of course, abundant evidence of genetic influences. In this study an attempt has been made to show a familial effect by asking 733 women attending a post-natal clinic, the breast-feeding history of their mothers. Of 397 women whose mothers had breast fed all children 60 per cent were fully breast feeding at eight weeks compared to 51 per cent and 43 per cent for women whose mothers breast fed some, or none of their children. The woman's attitude to breast feeding had a similar effect, but *her* mother's attitude to breast feeding seemed to be a negligible influence. This is a complicated subject, treated rather superficially. Without an intense and intimate study spanning at least two generations it is not possible to isolate environmental effects, such for example as the effect on a child at a very early age of seeing her mother breast feeding and many other subtle cultural influences. F. E. HYTTEN

Effect of Restricted Water Intake on Urine Nitrogen Output in Man on a Low Calorie Diet Devoid of Protein. F. Grande, J. T. Anderson, and H. L. Taylor, *J. Appl. Physiol.* 10:430, 1957.

Three groups of men were studied. One was allowed water *ad lib* while the others were permitted 900 and 1,800 ml/24 hr, respectively. Caloric intake was 1,000 cal in 24 hr. Vitamins and 4.5 g of NaCl/24 hr were also ingested. There was an expenditure of 120 cal daily because of walking. At the fifth day those on 900 ml put 9.4 g of nitrogen in 24 hr in the urine. However, if the intake of water was 1,800 ml then only 7.1 g of nitrogen was excreted. The subjects who had water *ad lib* put out 5.8 g. . Fecal nitrogen was unchanged, but sweat sometimes contained less than controls when water was restricted. Increase in nitrogen excreted and water deprivation were parallel. Blood urea nitrogen was elevated by the fifth day food was restricted and water allowed was 900 ml. At the time of dehydration there was a short period when the high nitrogen excretion continued. This represented 3 to 4 g of nitrogen which was washed out. The stress of dehydration produces increased nitrogen excretion as a metabolic response. Increased activity of the adrenal cortex plays a role in this response. Water is not thereby economized. Those on the smallest water allowance excreted the most urine.

M. J. OPPENHEIMER

Plasma-Insulin and Insulin Resistance. C. W. Baird and J. Bornstein. *Lancet* 1:1111, 1957.

A technic is described in some detail for the extraction of insulin from plasma. The activity of the extract and the residue was tested by the rat diaphragm technic. Using these methods, 22 persons were investigated and the results are presented briefly; seven were normal healthy subjects, the remainder were diabetic patients.

In healthy subjects after a meal the plasma insulin level is of the order of 1 to 2 milliunits per ml. Diabetics fall into two broad groups: those with and those without insulin in the plasma. Typically, two "juvenile diabetics" showed no insulin, but many had some insulin, in particular some in diabetic coma with insulin resistance. It is suggested that the activity of this insulin may be masked by antagonists.

F. E. HYTTEN

Chromic Sodium Chloride Toxicity: The Protective Effect of Added Potassium Chloride. G. R. Meneely, C. O. T. Call, and J. B. Youmans. *Ann. Int. Med.* 47:263, 1957.

The effect of chronic sodium chloride toxicity in the rat is the development of two kinds of hypertension. The first type seen at 2.8 and 5.6 per cent sodium chloride in the diet produces a clinical course similar to benign essential hypertension in the human, in that there is no excess of total body sodium and potassium does not affect the blood pressure. However, at higher levels of feeding (8.6 and 9.8 per cent NaCl) there is a further elevation of blood pressure which is reduced to an "intermediate level" by the addition of KCl. The latter also prevents an elevation of total body sodium observed in high salt diets.

This delightfully, at times whimsically, written article again calls attention to the interrelationships of Na and K and their role in the hypertensive state. There perhaps is some understanding evolved toward the reported effects of K in certain hypertensive patients. The authors point out that perhaps some humans, particularly those with heart disease, may be loading their diets with NaCl and concurrently depriving themselves of potassium. It certainly is food for thought!

J. F. MUELLER

Myonecrosis and Myoglobinuria in Alcohol and Barbiturate Intoxication. H. Fahlgran, R. Hed, and C. Lundmark. *Acta. Med. Scandinav.* 158:405, 1957.

A syndrome of pain, tenderness, and edema of the muscles is described following alcoholic excess or, in one patient, barbituric acid poisoning, in five chronic alcoholics. Histologic necrosis of the muscle was found in four patients, urinary myoglobin was demonstrated spectroscopically in three subjects and one case had a lower nephron nephrosis. A history suggesting recurrent attacks in relation to alcoholic bouts was sometimes elicited. The syndrome was associated with hemoconcentration, and hyperkalemia was thought to be the cause of death in two patients. The etiology of the condition is uncertain, and trauma was not thought to be responsible. W. H. J. SUMMERSKILL